Reducing Chilling Injury in Tomato:

Bridging the gap between cultivation and postharvest storage

Fahrizal Yusuf Affandi

Propositions

1. Colour and firmness de-synchronisation during cold storage and during subsequent shelf-life is a symptom of chilling injury in tomato.
   (this thesis).
2. Oxidative stress initiated by singlet oxygen ($^1O_2$) and the $^1O_2$-quenching potential of carotenoids likely determine the chilling tolerance of carotenoid-rich fruit such as tomato.
   (this thesis).
3. In any attempts to fight postharvest losses in the developing world, the loss of product quality, and not just quantitative product losses, should be taken into account.
4. Extreme focus on international accreditation obstructs progression in research for universities in developing countries.
5. Doing a PhD in the Netherlands means that you are also trained to become coffee lover.
6. In low income countries people need to get sweaty to earn money, in high income countries, people spend their money to get sweaty.

Propositions belonging to the thesis, entitled
Reducing Chilling Injury in Tomato: Bridging the gap between cultivation and postharvest storage

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Thesis

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To my late father, Bapak Endang Affandi (1955-2013),

and my mother, Ibu Ngesti Wahyuningsih

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CHAPTER

General introduction

1.1. Background

Temperature is the most important factor in extending shelf life and maintaining quality in horticultural products (Paul, 1999). Low-temperature storage has been considered to be the most effective method for maintaining the quality of many fruits and vegetables due to its effect on slowing down respiration, ethylene production, ripening, senescence, volatile alteration, and decay. In cold environment the physiological process in plants could be substantially retarded or even completely inhibited (Biswas, 2012). Temperature has a profound effect on the rates of biological reaction (e.g. metabolism and respiration) because enzymes which take part in this process are extremely temperature dependent (Nunes and Emond, 2003). It has also been proved that the growth and proliferation of microorganisms which cause rotting are limited at temperature around 0°C. Moreover, at the same relative humidity, weight loss resulting from moisture loss is reduced at low temperatures. However, instead of getting benefit from low temperature storage, some fruit and vegetable experience detrimental effects known as chilling injury (CI).

CI manifests as pitting, uneven and delay of colouring, development of brown areas in the peel, grainy and brown regions in tissues in the outer pericarp, and pathogen proliferation (Maa et al., 2014). CI is a disorder caused by prolonged storage at low temperature and occurs mostly in tropical and sub-tropical fruit and vegetables (Wang, 1994). Fruit such as mango, banana, papaya, guava, pineapple and tomato are chilling sensitive (Kader, 1999). Numerous intrinsic (e.g., cultivar, preharvest conditions) and extrinsic (e.g., temperature, exposure time to chilling temperatures, air humidity) factors influence severity of CI (Luengwilai et al., 2012). CI induced disorders reduces quality and consumer acceptability especially for fruit and vegetables grown in tropical and sub-tropical regions as well as hamper the possibility for transportation to an extended export destination. CI leads to substantial economic losses in the horticultural chain, especially to fruit and vegetables from tropical and sub-tropical origin (Wang, 1994). The FAO stated fruit and vegetable losses in South- and Southeast Asia can reach up to 51% with more than 35% of those losses during the postharvest and distribution stages of the supply chain (FAO, 2011).

Tomato (*Solanum lycopersicum*) is among the most produced and consumed crops in the world (FAO, 2014). However, post-harvest losses of tomato fruit can reach about 25-42% globally which represents not only quantitative but also qualitative losses (consumer acceptability and fruit nutritional content) (Arah et al., 2015). Tomato fruit quality is dependent on cultivar growing conditions and postharvest handling. The post-harvest life of tomatoes is limited by colour and firmness, and these two attributes determine the acceptance period of fresh tomatoes (Schouten et al., 2007). In Southeast Asian countries, tomatoes are usually harvested immature, at the mature green or breaker stage which allows storage and transport over longer distance (Chomchalow et al., 2002).

In tomato, CI symptoms are expressed as uneven ripening, surface pitting, excessive softening and increased susceptibility to fungal attack. It also affects properties such as loss of colour and flavour (Palash Biswas et al., 2012; Farneti et al., 2015; Zhang et al., 2016). Tomatoes suffer from chilling injury (CI) at temperature below 12 °C (Park et al., 2018; Luengwilai et al., 2012). However, negative tomato volatile alteration already occurs at temperature below 16 °C (Farneti et al., 2015, Maul et al., 2000). Chilling stress might also deregulate the normal ethylene-induced ripening process. However, climacteric ethylene is not essential for initiating CI in tomato, demonstrating that other factors are determinants in CI (Lurie et al., 1998, Luengwilai and Beckles, 2010).

In mango, CI symptoms are dark, scald-like discoloration and pitting or sunken lesions on the peel when fruit are stored at low temperature (below 13 °C) for long periods (Wang et al., 2008). Abnormal ripening and decay are typical CI symptoms. CI sensitivity of mango is cultivar dependant. For example, the difference in disorder such as peel browning of cold stored (4 °C) fresh mango fruits originated from Thailand could be attributed to difference in phenylalanine ammonia lyase (PAL) activity (Chidtragool et al., 2011), ascorbic acid content (AsA) and the activity of antioxidant enzymes such as super oxide dismutase (SOD) and catalase (CAT) (Chongchatuporn et al., 2013; Zhang et al., 2012). In banana, low temperature storage results in pitting and discoloration of the peel and abnormal ripening of the pulp (Murata., 1969; Wang et al., 2014; Luo et al., 2015). Browning in bananas is due to enzymatic oxidation of phenolics into quinones, which then polymerise into brown products (Nguyen et al., 2004). A correlation was found between chilling-induced peel browning and PAL and polyphenol oxidase (PPO) activity. Bananas treated with a hot-water dipping (HWD, 52 °C for 3 min) showed elevated chilling resistance (Wang et al., 2012).

1.2. Low Temperature stress induces CI in tomato

Cold storage induces numerous structural changes in cell structure that lead to disruption of several metabolic processes such as respiration and photosynthesis (Holtzapffel et al., 2003; Liu et al., 2012; İşeri et al., 2013). Several theories have been proposed to explain the primary cause of CI but the exact mechanisms and effect of these disorder is not yet understood (Sevillano et al., 2009; Luengwilai et al., 2012; Albornoz et al., 2019). In search for the primary cause of CI, primary or secondary injury events were considered. A primary injury events was defined as the initial rapid response to low temperature that causes a dysfunction in plant cells or metabolic processes (Aghdam and Bodbodak, 2014). This primary injury event is thought to be readily reversible if the temperature is brought back to non-chilling conditions. Sustained low temperature then leads to a cascade of processes that are considered secondary injury events, which may be irreversible and permanent whatever the subsequent handling of the product (Sevillano et al., 2009).

Alterations in plasma membrane lipid structure are thought to be the primary event that causes CI. As low temperature storage progresses, membrane lipid bilayers undergo transition from

liquid crystalline to a gel like structure. Historically, the initial hypothesis regarding the cause of CI is that low temperature causes physical changes in membrane lipid phase, conformation and hence fluidity which are important factor for proper membrane function (Lyons, 1973. Membrane solidification decreases membrane flexibility resulting in cracking and openings forming at the liquid-crystal/gel interface. These cracks and openings result in membrane leakiness, a loss of membrane integrity, and the loss of solute or ion gradients across the membrane (Raison et al., 1971).

The hypothesis on bulk phase changes of membrane lipid as a direct low temperature effect has been questioned (Sharom et al., 1994; Marangoni et al., 1996; Hodges et al., 2000). Hodges and Forney (2001) for instance, suggested that membrane damage occurs as a direct temperature effect, and is not visible until the product is brought back to a higher temperature. Previous studies have indicated lipid changes in mitochondrial membranes isolated from chilling-injured fruits, but none of the mitochondrial membranes showed phase transitions in chilling-resistant plants (Raison et al., 1971). However, later studies reported that during cold storage. Less than 5% of the lipid content changes to a gel like structure in most plants (Raison and Orr, 1986), and bulk phase transition was unlikely to happen in the biological membrane (Feigenson, 2007). Since there is a lack of direct observation of the phase change, the lipid phase change hypothesis is not convincing (Lukatkin et al., 2012). Therefore, development of lateral phase separation of which solid and liquid regions in a lipid bilayer at coexistence temperatures between the pretransition temperature and the transition temperature has been proposed (Liang et al., 2020). Avocado fruits stored at cold temperatures showed lipid phase separation in cold acclimated fruits, compared with fruit kept at 20 °C. However, this phase separation disappeared when these cold stored fruits were transferred to room temperature (Platt-Aloia and Thomson, 1987). This strongly indicates that the alteration of membrane properties is a gradual process depending on temperature-dependent physical properties of the membrane lipids, and that chilling-induced changes in the membrane can be reversed before irreversible damages in the membrane occurs (Mangaroni et al., 1996). Sharom et al. (1994) observed phase separation of chilled tomatoes only after 20 d of cold storage when they were brought back to room temperature for 5 d. Therefore, it was suggested that membrane phase separation took place only after occurrence of secondary events (for instance, lipid peroxidation) induced by prolonged low temperature storage (Marangoni et al., 1996). Lateral phase separations may be reversible until certain time and temperature combination where lipid degradation and accumulation of lipid degradation products induce irreversible membrane damage (Figure 1) (Sharom et al., 1994; Marangoni et al., 1996).

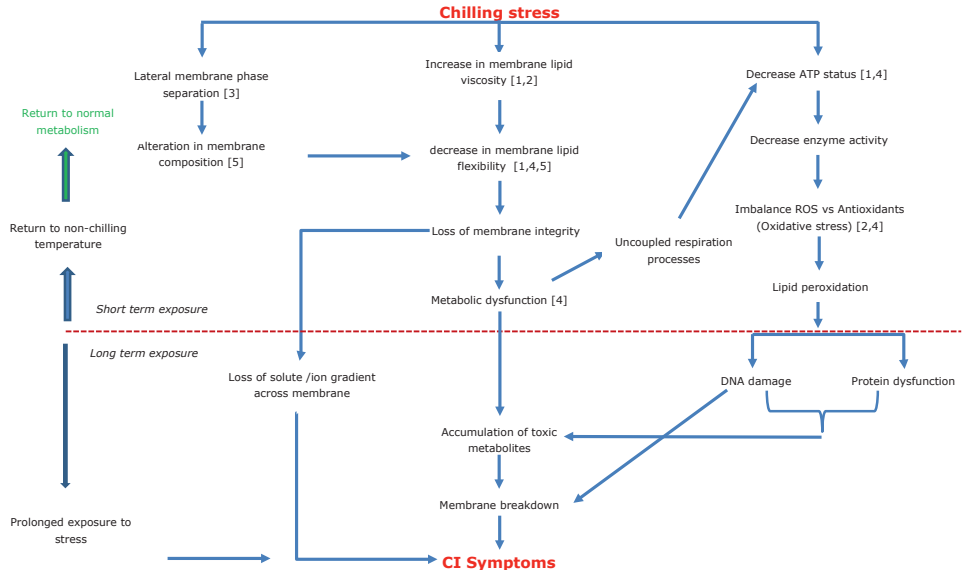

Figure 1. Schematic diagram of the initiation and development of CI in chilling sensitive produce. The number inside bracket indicates a process in which defence mechanisms might take place as indicated in figure 2 below. This figure was adapted from Lukatkin et al.(2012) and Aghdam and Bodbodak (2014)

In addition, careful observation indicated that events connected to membrane degradation are not independent events, instead they are interconnected. However, the order of each event is not clear. Nevertheless, studies pointed to one commonality: the plasma membrane is the early site of propagation of CI (Lyons, 1973; Platt-Aloia and Thomson, 1987; Parkin et al., 1989; Marangoni et al., 1996).

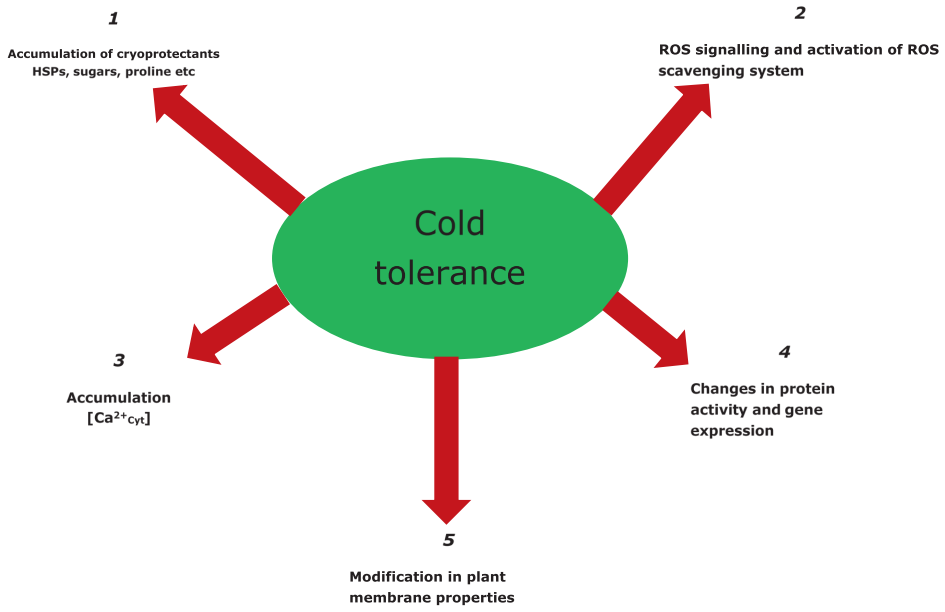

Figure 2. Different defence mechanisms induced as responses to cold stress adapted from Theocharis et al.(2012)

Low temperature stress induces alterations in the membrane composition. Around 80% of the membrane are made up of lipids (mainly phospholipids) and proteins (Larsson et al., 1990) with structural carbohydrates the remaining 20% (Murata and Nishida, 1990). Membrane composition, especially the type of lipids, greatly determine its physical characteristics and stability. Low temperature storage induces changes in lipid composition of the membrane of which MDGD (monogalactosyldiacylglycerol) and PC (phosphatidylcholine) are amongst the most sensitive membrane lipids to low temperature (Marangoni et al., 1996; Kong et al., 2018). In addition, the degree of unsaturation of the fatty acid side chains is closely correlated with the membrane fluidity and cold tolerance (van Meer et al., 2008). Many studies have revealed that cold-tolerance plants have a higher proportion of unsaturated fatty acids than cold-sensitive plants. The higher degree of unsaturated acids makes the membrane more liquid at lower temperatures. Unsaturated fatty acids have a lower melting point than saturated fatty acid owing to more double bound structure that therefore will not solidify upon cold storage (Lyons and Asmundson, 1965; Los and Murata, 1997). For example, "Qingzhong" fruits (a cold-resistant loquat cultivar) possess a higher content of unsaturated fatty acids than those in 'Fuyang' fruit, a cold-sensitive loquat cultivar (Cao et al., 2011).

Increasing the proportion of unsaturated fatty acid is thought to be an adaptive mechanism of plants to cold stress facilitated mainly by induction of fatty acid desaturase (FAD) (Vogg, 1998; Khodakovskaya et al., 2005). FAD maintains structure and function of membranes. The enzyme

creates the double bond C-Cs which allows for membrane fluidity upon low temperature stress (Los and Murata, 1998). Intermittent warming alleviated the CI of peppers largely because of increasing the unsaturated fatty acids content (Liu et al., 2015). In addition, the combination of salicylic acid and trisodium phosphate treatment enhanced fatty-acid desaturation efficiency indicated by the increased expression of key fatty acid desaturase genes, and higher content of unsaturated fatty acids (Ge et al., 2020). On the other hand, reduced levels of phosphatidic acid (PA) and diacylglycerol (DAG) may play a role in improving cold tolerance due its association with ROS production (Chen et al., 2015; Tan et al., 2018). In addition, monogalactosyldiacylglycerol (MGDG), a major chloroplast membrane lipid, and phosphatidylcholine sharply decreased during the CI development in green bel pepper, indicating they may be used as biomarker for cold tolerance (Kong et al., 2018). In addition, lipid composition of mitochondrial membranes is also affected by low temperature stress. Disruptions of the mitochondria can result in interference of metabolic processes that lead to the supply of ATP (Sevillano et al., 2009).

A decreased Adenosine Triphosphate (ATP) level in chilling injured fruit resulted in energy deficiency (Zhou et al., 2014; Pan et al., 2017). Prolonged cold stress resulted in reduction in ATP and adenosine diphosphate (ADP) levels in blueberries, and low ATP content was associated with increased pitting incidence under chilling stress (Zhou et al., 2014). Mitochondria and chloroplasts are the main sites involved in energy production to sustain the metabolism of a living cell. Alteration in lipid composition in the membranes can affect the function of mitochondria and chloroplasts during cold stress (Jing et al., 2009; Barrero-Sicilia et al., 2017). Cold stress may uncouple mitochondrial respiration as well as decreasing the activity of membrane bound ATPases leading to a significant drop in ATP production (Rurek et al., 2015). Similarly, low temperature also supressed the rate of photosynthesis resulting in a rigid thylakoid membranes and inactive enzymes (Liu et al., 2018). Disruption of these two energy-producing pathways because of the chilling injured membrane lead to metabolic disorder such as photoinhibition, enzyme inactivation, change in membrane fatty acid composition which in turn, increase the level of reactive oxygen (ROS) which is considered harmful to the cell membranes (Sevillano et al., 2009).

1.3. Oxidative stress, a key process that leads to CI

Apart from direct effect of low temperature on membrane integrity, oxidative stress likely is the secondary cause that initiates CI. Low temperature exerts abiotic stress to fruits that results in the inability to react to excess Reactive Oxygen Species (ROS) (Imahori et al., 2016). ROS such as singlet oxygen, hydrogen peroxide, superoxide anions, and hydroxyl radicals are the by-products of normal cell metabolism (i.e respiration and photosynthesis) (Mitler, 2002). Primary enzymatic sources of ROS in plant cells include photosystem II (Tijskens et al., 1994) and electron transport chain (Purvis and Shewfelt, 1993). Under physiological steady-state conditions, the plant responds to the presence of ROS by increasing the synthesis of antioxidants and scavenging enzymes (Purvis and Shewfelt 1993; Imahori et al., 2016). However, storage under

low temperature might uncouple the respiratory chain and initiate the production of considerable amount of ROS (Valenzuela et al., 2017). Low temperature also reduces the scavenging efficacy of enzymes and impairs the antioxidant turnover (Hodges et al., 2004; Imahori et al., 2016). The imbalance between ROS production and scavenging capacity is termed oxidative stress.

Excessive amount of ROS is dangerous because it can induce cell death (Mittler, 2002). ROS-attack membrane lipid and initiating membrane lipid peroxidation. Lipid peroxidation is a natural process in plants and increases during plant senescence or ripening, however, it can also increase as a consequence of oxidative stress caused by chilling injury (Lado et al., 2015). Accumulation of peroxidised lipids leads to phase changes of the lipid bilayer membrane from a liquid crystalline to a gel like structure (Apel and Hirt 2004; Hodges et al., 2004). As the membrane solidifies it becomes less flexible. Cracking and channels can form at the liquid-crystalline to gel interface leading to membrane leakiness, solute loss, and membrane integrity loss (Hodges et al., 2004). ROS may also attack proteins in the membrane, and finally DNA and RNA leading to an accumulation of toxic metabolites, metabolic disorders and finally cell death (Liang et al., 2020). Alternatively, enhanced ROS levels especially hydroxyl radicals ($OH^{\cdot}$) can activate programmed cell death (PCD) pathways (Gechev et al., 2006). Exposure of tobacco BY-2 suspension cells to 5–6 °C for two to 5 weeks resulted in characteristic features of PCD, including DNA condensation and fragmentation (Koukalova et al., 1997). In cucumber chilled at 2 °C, characteristic features of PCD were induced at the same of time (9d) of CI initiation (Zhao et al., 2014).

1.4. Multiple pathways are involved in chilling tolerance

Defence mechanisms to limit oxidative stress are governed by both enzymatic and non-enzymatic antioxidant species (Gill and Tuteja, 2010). Non-enzymatic compounds include ascorbic acid (AsA), glutathione, proline, polyamines, carotenes, flavonoid, and α-tocopherol (Demidchik, 2015). Enzymatic compounds comprise SOD, CAT, peroxidases (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) (Hodges et al., 2004). AsA, glutathione, and α-tocopherol work synergistically. GSH (a reduced form of glutathione) is used by GR to regenerate AsA from dehydroascorbic acid (DHA) and AsA can regenerate α-tocopherol from its oxidized form (Hariyadi and Parkin, 1991). Moreover, AsA is the most abundant antioxidant in plant cell which also serves as electron donor to many important reactions (Szarka et al., 2012). In terms of enzymatic antioxidants, the balance between SOD, CAT and APX is crucial to maintain a low level of superoxide anion and hydrogen peroxide. Together with sequestering metal ions, this balance is important to avoid generation of hydroxyl radicals ($OH^{\cdot}$) which are the most reactive and toxic ROS (Mittler, 2002). Cold stress has been shown to increase H_2O_2 accumulation in cells and to enhance the activity of antioxidant enzymes and transcript levels of the genes encoding them (Kuk et al., 2003).

In tomato, special attention is paid to lycopene, a carotenoid which possesses the highest singlet oxygen scavenging capacity (Di Mascio et al., 1989). The antioxidant capacity of lycopene is contributed by its eleven conjugated double bonds (Llansola-Portoles et al., 2015). One of the symptoms of CI is lycopene degradation in red ripe tomato (Farneti et al., 2012). Lycopene also protects other carotenoids from oxidative stress. Upon contact with ROS, lycopene may undergo isomerisation or degradation (Heyman et al., 2015). Lycopene degradation does not only reduce tomato nutritional value, but also decreases its visual quality since lycopene is the main tomato colour pigment and one of the most important quality factors perceived by consumers (Schouten et al., 2007). Besides colour, aroma volatile production is often affected at CI temperatures. Some volatiles are increased due to lipid peroxidation and lycopene breakdown linked with a negative consumer perception (Farneti et al., 2014). Nair et al. (2003) reported a significant reduction in total aroma volatiles, monoterpenes, sesquiterpenes, esters, aldehyde, and nor isoprenoids for tomatoes stored at chilling temperatures. This loss is associated with significant reduction in the transcripts of genes encoding for key enzymes in volatile biosynthesis. Recent work demonstrated this is caused by a cold-induced change in methylation status of the promoters of these genes. After a return to 20 °C, the expression of these genes did not return to pre-chilling transcript levels (Zhang et al., 2016).

Apart from antioxidants, also other mechanisms are thought to function as a response to chilling stress (Figure 2). One of them is a C-repeat/dehydration-responsive element (CRT/DRE)-binding factor (LeCBF1), a transcription factor regulated by exogenous ethylene. CBF is expressed following cold induction, and is associated with cold injury protection (Zhao et al., 2009; Zhao et al., 2011). The CBF family consist of CBF1, CBF2 and CBF3. Overexpression of CBF1 in Arabidopsis induces the expression of a wide range CBF/DREB-regulated COR genes and to enhance whole plant freezing tolerance without a low temperature stimulus (Jaglo-Ottosen et al. 1998; Liu et al., 1998; Maruyama et al., 2004). The three CBF genes are induced within 15 min after exposure to low temperatures, and accumulation of targeted CBF/DRE-regulated COR genes starts within 2 h (Mantyla et al., 1995). CBF transcription factors activate a cluster of genes that are collectively referred as the CBF regulon. Among these genes, the most important members are those belong to cold regulated gene (CORs) family, such as low-temperature induced (LTI), cold-inducible (KIN), and responsive to desiccation (RD) family and the dehydrin (DHN) family. Abscisic acid and dehydration also induce CBF genes expression (Shinozaki and Yamaguchi, 2000; Knight et al., 2004). Many CBF homologues have been found in higher plants both cold tolerant and cold sensitive (Ruelland et al. 2009; Zhao et al., 2009).

Heat shock proteins (HSPs) are also reported to play a role in cold tolerance (Lurie et al., 2003). Heat shock effect can be achieved through different methods for instance by holding the fruit at higher temperature (38 °C), hot water dipping, or hot water spraying over the fruit (Rodriguez et al., 2005; Luengwilai 2012; Zhang et al., 2012). Moderate heat stress (heat shock treatment) for a limited time (i.e. hot water treatments) after harvest induces production of small Heat Shock Proteins (sHSPs) that contributes to acquiring chilling tolerance (Lurie, 1998). HSPs convers

protection to stress due to their chaperone activity which consists of (i) ability to recognise and bind to unfolded proteins in order to complete their folding correctly, (ii) preventive action against protein aggregation, and (iii) the contribution to renaturation of aggregated proteins (Aghdam et al., 2013) Moreover, HSPs and HSTFs (Heat shock transcription factors, that regulate HSPs synthesis) are able to sense ROS and thus activates defence mechanism to overcome oxidative stress (Zhang et al., 2005). In addition to its chaperone activity, small HSPs (sHSPs) posses ability as membrane stabilizers and ROS scavengers which act synergistically with antioxidant system. sHSPs induce accumulation of antioxidant enzymes such as APX, CAT, and POX to prevent photoinhibition and thylakoid degradation in transgenic tobacco plants exposed to chilling (Li et al., 2012). The tomato chloroplast sHSP, HSP21, is induced by heat treatment in developing fruits during the transition of chloroplasts to chromoplasts which protects photosystem II from temperature-dependent oxidative stress (Neta-Sharir et al., 2005). A transcriptomic study targeting the RNA-seq at mature-green 'Micro-Tom' tomatoes exposed to a heat treatment of 40 °C for 7 min followed by long-term cold storage 5 °C for 14 d, revealed that chilling tolerance is mediated by up-regulation of heat shock transcription factors, heat shock proteins and genes that code for proteins involved in detoxification (Cruz-Mendívil et al., 2015).

1.5. Preharvest factors affect CI sensitivity

Cultivation practices affect the sensitivity of fruit to chilling injury (Ferguson et al., 1999). It is believed that moderate stress such as temperature, water status or light during cultivation trigger plants to react by initiating immediate protection against the stressor. After being exposed to the initial stressor the plant will now also be protected against other stresses simultaneously or subsequently (Neta-Sharir, 2005; Lurie, 2004 and Jiang, 2002).

Exposure to high temperatures on the vine, particularly close to or at harvest, may induce tolerance to low temperatures (Ferguson et al., 1999). Avocado fruit exposed to direct sunlight on the tree, frequently exceeding 35 °C, showed lower CI levels than fruit from shaded parts of the tree when stored at 0 °C. In addition, cucumber fruit grown in elevated greenhouse temperature (32 °C) improve their tolerance to chilling (less weight loss, no visible symptoms of CI and lower ion leakage) than fruit grown at control temperature (27 °C) due to maintenance of higher firmness and enhance activity of antioxidant enzymes such as SOD and CAT (Kang and Saltveit, 2001). On the other hand, grapefruit, sweet pepper and shoots of sweet basil have been shown to be less sensitive to chilling temperatures when exposed to temperatures between 12 and 18 °C (Ferguson et al., 1999). Exposing plants to sub-optimal growing temperature between 10 – 20 °C for certain duration (7 – 10 days) induces tolerance to chilling in tomato plants (Barrero-Gil et al., 2016); watermelon (Lu et al., 2020) and sweet pepper (Ferguson, 1999). Increased tolerances to low temperature (4 °C) of tomato plants and watermelon fruit were mediated by thorough transcriptomic and metabolic adjustment occur at two steps. First, early responses involving transient changes in gene expression of stress related proteins and hormone signalling such as CBFs genes, and hormones like ethylene, abscisic acid (ABA) and gibberellin

(GA). Second response after 24 h including stable gene expression encodes full metabolic adjustment such sugar accumulation, amino acid accumulation, enhancement of antioxidant mechanism (accumulation of flavonoids and anthocyanin) and re-arrangement of photosynthetic machinery.

Light is an environmental factor that also influences chilling sensitivity (Wang et al., 2019; Hoffman et all., 2015). Light treatments can be given during preharvest or postharvest stage to induce cold tolerance (Ahres et al., 2020). Stress tolerance (including cold tolerance) improvement by light might be mediated by enhancement of antioxidants level, activation of CBF defence pathways and increased sugars accumulation (Joung-Kim et al., 2002; Maibam et al., 2013; Bianchetti et al., 2018). The light spectrum, intensity and duration of light treatments are factors that influences the production of antioxidants and other secondary metabolites affecting the oxidative stress levels (D'Souza et al., 2015). In addition, light induces sugars accumulation which in many cases are considered as cryoprotectants, osmoregulators and signalling molecules that provide tolerance to cold stress. Sugars convers protection to plant cell membranes during cold-induced dehydration by replacing water molecules and form hydrogen bonds with lipid molecules (Uemura et al. 2003; Ruelland et al. 2009). Moreover, carbohydrates may also act as ROS scavengers that stabilises the membrane upon cold stress. Sugar signalling is also closely associated with hormone signalling, the control of growth and development, and stress responses in plants (Zeng et al. 2011).

Far-red (FR) lighting is known to induces many morphological responses in plants, including promotion of shoot elongation and reduction of stem diameter in dicotyledonous and ornamental species from a low R:FR (Kalaitzoglou et al., 2019). Apart from the morphological effect, far-red (FR) light positively and Red (R) light negatively regulates cold tolerance in tomato plants during cultivation (Wang et al., 2016). FR induced activation of PHYA and subsequent ABA and JA signalling leading to activation of the CBF stress signalling pathway genes leading to chilling tolerance in tomato leaves (Wang et al., 2016). In the postharvest stage, FR lighting stimulate changes in cuticle wax composition resulted in a tighter cuticle-wax adhesion and enhanced barrier properties against transpiration (Cozmuta et al., 2016). Transpiration is also thought to be associated with the decline of MGDG and PC membrane lipids in bell pepper, and can be a good indicator of CI (Cohen et al., 1994; Maalekuu et al., 2006; Kong et al., 2018).

Postharvest blue light (BL) increases antioxidant enzymes activity as well as the content of antioxidants such as AsA and tocopherol in strawberry (Xu et al., 2014). Accumulation of anthocyanins in lettuce (Li and Kubota, 2009), Chinese cabbage (Avercheva et al., 2014), and grape berries (Kondo et al., 2014) were also reported as an effect of BL illumination. Postharvest illumination of BL for seven days resulted in tomato surface yellowing and firmness retention due to an increase γ-aminobutyric Acid (GABA) and decrease glutamic acid (GLU) production which negatively correlated to ripening (Dhakal and Baek, 2014).

Apart from FR and BL lighting, UV-C irradiation was also reported to alleviate CI. UV-C treatment (7 kJ m^{-2}) on peppers reduced decay incidence, weight loss and maintain higher firmness after 10 d of cold storage at 10 °C followed by 8d shelf life at 20 °C (Vincente et al., 2005). Three to ten minutes of UV-C irradiation with an intensity of 8.22 W m^{-2} reduced chilling injury significantly after storage at 5 °C plus 7 days of shelf life at 20 °C as well as a reduction in fungal decay in peaches (Gonzalez-Aguilar et al., 2004). Gonzalez-Aguilar et al. (2007) attributed the efficacy of UV treatment to its ability to induced accumulation of phenols and flavonoids which was positively correlated with antioxidant capacity.

Water deficit induces production of ABA and increases antioxidant enzyme activity such as SOD, CAT, APX and GSR (Jiang and Zhang, 2002). Water deficit also induces dehydrin production, proteins that are suggested to have protective effects against water and temperature stress. Dehydrin genes are expressed under regulation of ABA-dependent and ABA-independent signalling pathways (Hanin et al., 2011). Dehydrins act as radical scavengers leading to a reduction of lipid peroxidation thereby increasing cold tolerance of tobacco (Hara et al., 2003).

1.6. The technology behind low oxygen storage alleviation of CI

Controlled atmosphere (CA) and modified atmosphere packaging (MAP) are storage and packaging technologies that limit respiration through manipulation of O_2 and CO_2 levels that differ from the normal atmosphere (20–21% O_2, about 0.03% CO_2, about 78–79% N_2, and trace quantities of other gases) to delay ripening and senescence of fruit and vegetables (Sozzi et al., 1999; Brandenburg and Devon, 2009). CA and MAP involve atmospheres with reduced oxygen or elevated carbon dioxide (Saltveit, 2003). The difference between CA and MA technology relies upon control and management to achieve desired atmospheric condition (Fagundes et al., 2015). In CA, the atmosphere condition is achieved and controlled strictly to be as close as possible to the set point. On the other hand, MAP does not apply a strict atmospheric control as CA. In MAP the required atmosphere condition is achieved by physiological processes e.g., respiration (passive MAP), or combined with flushing the packaging container with single or mixed gas during packaging (active MAP) (Martínez-Romero et al., 2003). Therefore, once the produce is put inside MAP, atmosphere condition is no longer controlled or modified by measuring gas concentration or applying active gas modification (Hoehn et al., 2009)

Low oxygen inhibits CI incidence due to restricted oxygen availability for peroxidation of cell membrane lipids, reduces oxidative stress by lowering ROS levels and by maintaining higher levels of antioxidants such as ascorbic acid, and carotenoids (Brandenburg and Devon, 2009; Fahmy and Nakano, 2014).

Lowering the availability of the oxygen causes a decline in a cell's general metabolism. Reduction of oxygen decreases respiration, ripening, softening, senescence, decomposition of sugars, acids, pectins, and proteins. Low oxygen decreases ethylene production by fruit and vegetables and decrease their sensitivity to ethylene action (Kader, 1986). This is because the

synthesis of both 1-amino-cyclopropane-1-carboxylic acid (ACC) and ACC-synthase requires oxygen (Gorny and Kader, 1996). The optimum oxygen concentration for storage varies due to difference in the rate of oxygen consumption and diffusion and temperature. On the other hand, a too low oxygen concentration leads to shifting from aerobic respiration to fermentation which resulted in ethanol and acetaldehyde production that can lead to off-flavour (Kanellis, 2009).

Recent findings reported that low oxygen stress to some extent, provides beneficial effects for fruit to cope with cold stress (Cukrov et al., 2019). Low concentration of ethanol in the fruit tissue (depends on species and varieties) is not only acceptable in a sense that it does not contribute to off flavour but also may induce positive effects (Yahia 2009; Pesis, 2005; Hodges et al., 2004;). Exogenous ethanol treatment was reported to be a major ROS scavenging natural volatile in tomato (Yanuriati et al., 2009). At the same time, in cucumber it was suggested to induce cold tolerance by membrane-lipid fluidization (Sabban-Amin et al., 2011). Amino acid metabolism is also affected by low O_2 storage. The concentration of alanine, aspartate, aminobutyric acid (GABA), proline, serine, and threonine, has been found often to be modulated by oxygen level during fruit CA storage (Cukrov et al., 2019). GABA and proline are known to have protective effect on membrane integrity (Aghdam et al., 2012; Zhang et al., 2013; Dhakal and Baek, 2014). Hydrogen peroxide accumulates under low oxygen stress and is thought to act as signalling molecule that triggers several defence mechanisms against prolonged stress such as heat shock protein transcript factor (HSFs), heat shock proteins (HSPs), and enhancement of antioxidant enzymes (Pegoraro et al., 2012). MAP application has been shown to alleviate CI in fruit due to the maintenance of moderate to high relative humidity (RH) inside the package (Batu and Thompson, 1998; Zainon et al., 2004).

1.7. Aim and content of this thesis

The aim of this thesis is to elucidate the role of several preharvest and postharvest factors that contribute to chilling tolerance. The thesis studies the role of preharvest factors such as light and temperature during growth and aims to explain physiological basis behind CI and chilling tolerance. This thesis also investigates the processes that lead to CI and tackles the disruptive effects of CI on tomato quality by applying low oxygen storage. Moreover, interaction between preharvest lighting with low oxygen storage in minimising CI is also investigated.

In **Chapter 2**, the role of preharvest FR lighting on cold tolerance is demonstrated. The FR effect on cold tolerance was tested by employing combination of FR lighting and variation in cold durations to alter the chilling stress. It was shown that FR affects the synchronisation between firmness and colour at harvest. FR cultivated red tomato was firmer at harvest and this effect was an important feature that contributes to better cold tolerance. It was also shown that mature green harvested tomatoes, cultivated with additional far-red light, had lower CI incidence expressed as reduced weight loss, less pitting, faster red colour development during shelf life, and less softening. In Chapter 2, it is shown that cold tolerance can also be improved by a

cultivation factor (lighting) and chilling injury phenomenon can be assessed by quality properties behaviour analysis during shelf life storage employing non-destructive techniques.

The role of preharvest blue LED lighting (BL) on CI alleviation is described in **Chapter 3**. With an almost similar setup with experiment in chapter 2, it is demonstrated that BL lighting in tomato cold tolerance is mediated by lower maturity at harvest and higher red colour loss during cold storage, which points to higher lycopene utilisation to fight oxidative stress. The effect of BL on several antioxidant (AsA and CAT) and oxidative stress indicator (MDA and H_2O_2) was also studied, and it was reported not to be related with CI incidence. Taken together with lycopene result, this point to significant variation in defence pathways that are activated in response to cold storage.

In **Chapter 4** the possibility of temperature stress during cultivation is explored and possible mechanism underlying each factor is hypothesised. The role of growing factor and cold storage on colour development is also described. We found that growth at lower temperature than standard growth temperature induced cold tolerance and this is connected to retention of firmness of lower weight loss. Colouring delay was also observed which implies the involvement of lycopene or lycopene precursor in cold tolerance. This effect was cultivar dependent.

In **Chapter 5**, the effect of low oxygen on CI was tested on commercial cultivar through different set of experiment. In the first experiment, the low oxygen range from moderate low (5%) to extremely low (0.5%) effect on CI development were tested. The effect of maturity was tested by using two maturities: mature green and red. In the later experiment, the effect of combining low oxygen storage and FR lighting was tested. Storage under reduced partial pressure of O_2 reduced CI incidence in both MG and R tomatoes. Lower oxygen uptake was shown for MG tomatoes stored at 1 kPa O_2 previously cultivated with FR. This might later facilitated reduction in singlet oxygen formation and maintain colour synthesis.

In **Chapter 6**, General Discussion, the result in chapter 2-5 are put into more physiological aspect. The limitation of the current studies, practical implication as well as further research possibilities are envisaged and discussed.

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CHAPTER

2

Far-red light during cultivation induces postharvest cold tolerance in tomato fruit

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Abstract

We investigated the role of far-red LED light during cultivation on postharvest cold tolerance in tomato fruit (*Solanum lycopersicum* cv Moneymaker). Red and blue top LED light, providing 150 μmol m^{-2} s^{-1} photo-synthetically active radiation (PAR) at plant height for 16 h daily, was combined with 0, 30 or 50 μmol m^{-2} s^{-1} (non-PAR) far-red LED light. Tomatoes were harvested at the mature green or red stage and subjected to cold storage for 0, 5, 10, and 15 d at 4 °C, followed by 20 d shelf life at 20 °C.

Mature green harvested tomatoes, cultivated with additional far-red light, showed reduced weight loss, less pitting, faster red colour development during shelf life (when prior long cold stored), and less softening (when prior short or non-cold stored). FR lighting during cultivation likely protects the membrane integrity of MG tomatoes and thus allows uninterrupted lycopene synthesis. Red harvested tomatoes cultivated with additional far-red light were firmer at harvest, showed reduced weight loss and less decay during shelf life. Less red colouration was observed for red harvested fruits at the start of shelf life when fruits were prior cold stored, indicative of lycopene breakdown during cold storage. The improved cold tolerance of red harvested fruits grown under additional far-red light is likely due to higher firmness at the start of the shelf life period with lycopene acting as antioxidant during cold storage. In conclusion, additional far-red light during cultivation improved postharvest cold tolerance for tomatoes harvested at both the green and red maturity stage, and might therefore be suitable to prolong the storage potential of tomato at sub-optimal temperatures.

2.1. Introduction

Chilling injury (CI) is a physiological disorder induced by exposure to low, but above freezing temperatures. Chilling-sensitive crops are of tropical and subtropical origin and includes tomato (Lurie and Crisosto, 2005). CI is described as a reversible response to low temperature which causes a cascade of secondary effects that results in CI symptoms (Biswas et al., 2017). Symptoms of CI in tomato include uneven ripening, surface pitting, excessive softening and increased fungal decay. CI also affects quality properties, such as loss of colour and flavour (Biswas et al., 2012; Farneti et al., 2015; Zhang et al., 2016). These detrimental changes reduce consumer acceptance leading to substantial economic loss especially for fruit grown in tropical and sub-tropical regions due to inability to maximise the benefit of low temperature storage (Wang, 1994).

A major cause of CI is considered to be oxidative stress (Shewfelt and Del Rosario, 2000). Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the capacity of the fruit to maintain cellular redox homeostasis (Aghdam and Bodbodak, 2014). Oxidative stress results in lipid peroxidation and affects membrane integrity (Hodges et al., 2004) which results in visible CI signs (Malacrida et al., 2006). Farneti et al. (2012) found that red tomatoes experienced lycopene loss during cold storage, thereby indicating that lycopene might scavenge excess ROS (Gartner et al., 1997; Stahl and Sies, 2003). Cold tolerance is typically characterised by increased activity of antioxidant enzymes and lower ion leakage. Cold tolerance can be induced in tomato fruit by methyl jasmonate and methyl salicylate (Ding et al., 2001), gibberellin (Ding et al., 2015) and glycine betaine in pepper fruit applied as pre-chilling storage dips (Wang et al., 2016). Intermittent warming has been applied during cold storage and was effective in delaying CI symptoms in tomato, but was highly cultivar dependant (Biswas et al., 2012). Heat shock treatments by immersion of tomato fruit in 40 °C water for 7 min reduced CI of fruit stored for 14 d at 2.5 °C (Luengwilai et al., 2012). These treatments are all applied after harvest, and hardly any attention has been paid to growth conditions that may induce cold tolerance in tomato fruits.

Far-red (FR) light during cultivation increased total plant dry mass, fruit yield, fruit number per plant, fruit weight per plant and average fruit weight (Zhang et al., 2018; Kalaitzoglou et al., 2019). Far-red light was shown to affect cold tolerance in tomato plants during cultivation. A low R/FR ratio stimulated ABA and JA biosynthesis leading to activation of the C-REPEAT BINDING FACTOR stress signalling pathway genes, leading to cold tolerance in tomato plants (Wang et al., 2016). It is unknown whether FR light can also induce cold tolerance in tomato fruit. We investigated whether additional FR LED lighting during cultivation affects cold tolerance of harvested tomato fruits. Tomato 'Moneymaker' plants were grown at three FR levels. Fruits from the three FR treatments were harvested at the same colour stage, either mature green (MG) or red (R). These tomatoes were subsequently stored for either 0, 5, 10, or 15 d at 4 °C followed by a shelf life period of 20 d at 20 °C. Chilling injury indices, weight loss-, colour- and firmness behaviour were assessed during shelf life. We show that additional FR

light during cultivation induced CI tolerance. The physiological mechanisms involved, and benefits of applying FR lighting in the tomato supply chain are discussed.

2.2. Materials and methods

2.2.1. Plant material and growth conditions

Tomato (*Solanum lycopersicum* cv Moneymaker) seeds were sown on November 28th, 2016 and germinated under natural light. Uniform seedlings were transplanted on December 15th, 2016 into 7.5 litre pots and placed in a greenhouse compartment at Wageningen University (52° N, 6° E, Wageningen, the Netherlands). Day/night temperature was maintained at 22/18 °C until plants started flowering (35 days after transplanting). Thereafter, temperatures were adjusted to 20/16 °C to facilitate fruit set. Daily average relative humidity was maintained at 78 ± 5%. The plants were irrigated with a nutrient solution (electrical conductivity 2.1 dS m^{-1}, pH 5.5). Further greenhouse management (fertigation, pollination) was according to commercial practises. Light treatments were separated by double sided, non-transparent, white reflective plastic sheets.

2.2.2. Light treatments

Supplementary lighting was provided by RB (Red+Blue) and FR LED modules (RB: GreenPower LED -TL-DR/B-150, FR: GreenPower LED -PM-FR-150, Philips, Eindhoven, the Netherlands). The height of the LED modules was adjusted weekly to maintain the desired photo-synthetically active radiation (PAR) at the top of the canopy to 150 μmol m^{-2} s^{-1}, constant for all light treatments. When the LEDs reached the maximal height of the greenhouse, the top of the canopy was lowered weekly, as is usual in a high wire cultivation system. A spectroradiometer was used to ensure that both light intensity and the phytochrome photo equilibrium (PSS) values were kept constant. The intensity of the (non-PAR) FR LED light consisted of additional 30 (FR30) or 50 μmol m^{-2} s^{-1} (FR50), next to a control with no additional FR light (FR0). The treatments affected the PSS values from 0.88 (FR0) to 0.84 (FR30) and 0.80 (FR50). Photoperiod was set to 16 hours. On average, solar photosynthetically active radiation (PAR) contributed 12% to the total PAR integral during the whole experiment at canopy level, indicating that the LED light provided the majority of the incident PAR to the tomato plants. The spectral composition of the light treatments is shown in Figure 1.

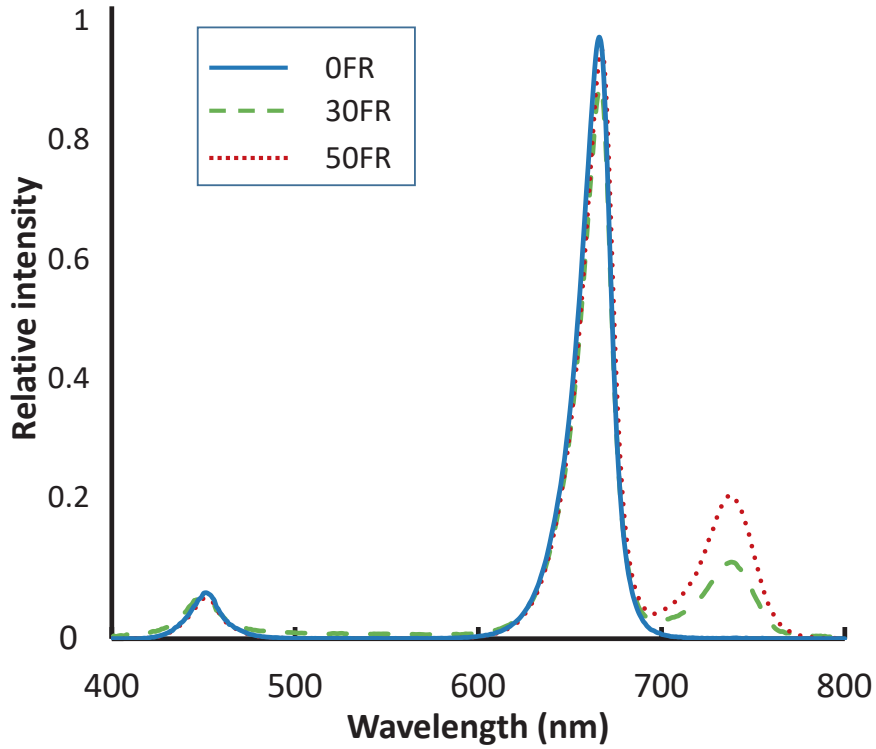

Figure 1. Spectral distribution of the light treatments: 150 µmol $m^{-2} s^{-1}$ Red and Blue LED light (FR0), 150 µmol $m^{-2} s^{-1}$ Red and Blue LED light with 30 µmol $m^{-2} s^{-1}$ FR LED light (FR30) and 150 µmol $m^{-2} s^{-1}$ Red and Blue LED light with 50 µmol $m^{-2} s^{-1}$ FR LED light (FR50).

2.2.3. Fruit selection, colour measurements and storage conditions

Mature green (MG) and red (R) tomato fruits were harvested on April 18, 2017. Directly after harvest, colour was measured on three equatorial positions of each tomato by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany). Remittance was assessed at 570 (R570), 660 (R660) and 780 (R780) nm by calculating the normalised anthocyanin index (NAI) (Eq. 1) and normalised difference vegetation index (NDVI) (Eq. 2) which are both normalized value between −1 and 1 (Schouten et al., 2014).

$$NAI = \frac{R780\text{-}R570}{R780+R570} \quad (1)$$

$$NDVI = \frac{R780\text{-}R660}{R780+R660} \quad (2)$$

Per light treatment and per maturity stage, twenty tomatoes were selected that showed NAI values between -0.7 and -0.5 for MG and between 0.3 and 0.5 for R tomatoes, in total 120

tomatoes. Tomatoes were subsequently marked on two positions on the equator for repeated colour and firmness measurements over time. Twenty tomatoes per light treatment and maturity stage were randomly assigned to assess the quality attributes at harvest. Five randomly assigned tomatoes per light and maturity stage were stored for either 5, 10 or 15 d at 4 °C in the dark followed by 20 d at 20 °C. Firmness and colour measurements were started immediately after the cold period and were carried out approximately every two days during shelf life. In addition, weight and two CI indices were assessed approximately every two days during shelf life.

2.2.4. Firmness measurements

Non-destructive stiffness was measured using a commercial acoustic firmness tester (AFS, AWETA, Nootdorp, the Netherlands) with the tick power of the plunger set to 15. The AFS combines the single tomato resonant frequency (f in Hz) and mass (m, in kg), measured by an inbuild balance, into a FI (firmness index, Eq. 3) (Schotte et al., 1999; Schouten et al., 2018).

$$FI = \frac{f^2 m^{2/3}}{10^4} \tag{3}$$

2.2.5. CI indices and weight loss

Vega-García et al. (2010) introduced a chilling injury index that included uneven ripening, surface pitting and decay. We have adapted this chilling injury index into a pitting and a decay index. This was done as MG tomatoes were only affected by surface pitting and red tomatoes were only affected by decay symptoms, such as the presence of fungal infections or skin lesions. Both indices were visually assessed with the percentage of the affected tomato surface assigned to five classes (0= no injury, 1= < 10%, 2= 11-25%, 3= 26-40%, 4= > 40%). Tomato weight loss was expressed as the percentage weight loss (WL) with W_0 the initial weight (in g) and W_t the weight (in g) at time t (Eq. 4).

$$WL = \frac{W_0 - W_t}{W_0} \cdot 100 \tag{4}$$

2.2.6. Statistical analysis

Data measured at harvest were subjected to one-way ANOVA and data obtained during shelf life were subjected to mixed ANOVA, applying SPSS ver.21 (SPSS, Chicago, USA) at $P < 0.05$. Mixed ANOVA was applied with light treatment and cold duration as between subject factors and shelf life days as within subject factor. Normality of the variables was tested applying the Shapiro-Wilk test. Mauchly's test of sphericity was carried out to test whether variances of the differences between all possible pairs of within-subject conditions were equal. If the sphericity assumption was not fulfilled, Greenhouse-Geisser's correction was applied to calculate the

degrees of freedom. In case of a significant interaction, a pairwise comparison was carried out for each shelf life day with LSD (Least Significant Difference) values estimated.

2.3. Results

Two chilling injury indices, colour, firmness and weight loss data of tomatoes, gathered during a shelf life period of 20 d that had been prior cold stored for either 0, 5, 10 or 15 days are shown here. Data are presented for twenty MG and R tomatoes at harvest or five MG and R tomatoes measured repeatedly during shelf life.

2.3.1. Additional far-red light during cultivation lowered chilling induced pitting in mature green tomatoes during shelf life

MG tomatoes that were not cold stored showed minor pitting development during shelf life (Figure 2A). Pitting was slightly higher for MG tomatoes that were prior cold stored for five days (Figure 2B). Prolonged cold storage (10 and 15 d) resulted in high pitting index values for MG tomatoes at the start of shelf life with lower values for FR50 tomatoes. Remarkably, the pitting index decreased during shelf life, irrespective of light treatment. Following prolonged cold storage, the pitting index values during shelf life were always lower for FR50 compared to FR0 tomatoes (Figure 2C, D).

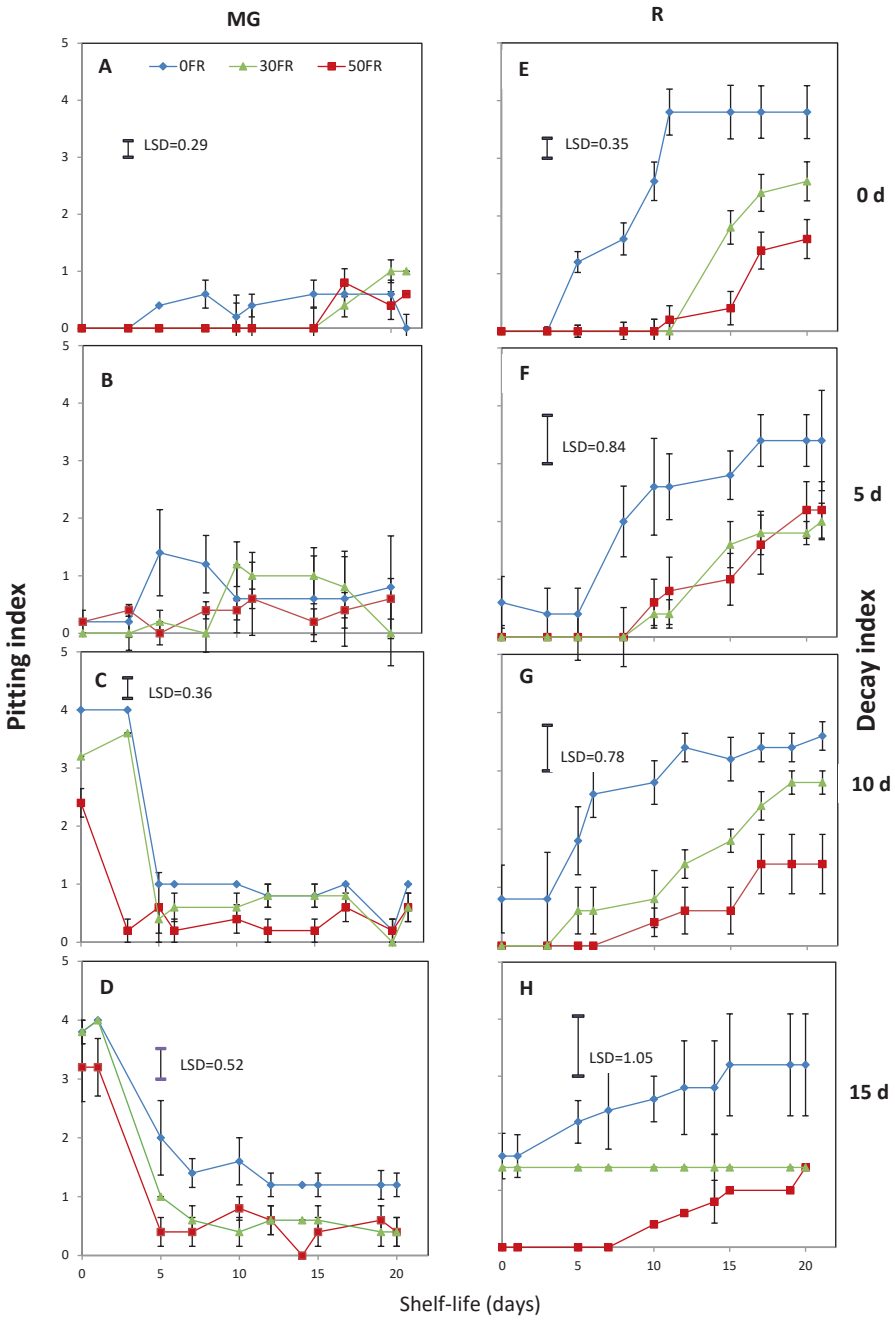

Figure 2. Pitting index for mature green (MG) harvested and decay index for red (R) harvested tomatoes during shelf life expressed for three light treatments: without (FR0) and with either 30 (FR30) or 50 µmol m^{-2} s^{-1} (FR50) additional FR light. The average pitting index with indicated standard error is shown for

five MG tomatoes during shelf life at 20 °C (A) and after a cold storage at 4 °C for 5 d (B), 10 d (C) or 15 d (D). The average decay index with indicated standard errors is shown for five R tomatoes during shelf life at 20 °C (E) and after a cold storage at 4 °C for 5 d (F), 10 d (G) or 15 d (H). LSD values ($P < 0.05$) are indicated per plot. Absence of LSD bars is indicative of no significant differences between light treatments.

2.3.2. Additional far-red light during cultivation lowered chilling induced decay in red tomatoes during shelf life

With no cold storage applied, the decay index for R tomatoes started to increase after 5 d of shelf life in tomatoes cultivated with no additional far-red light (FR0) whereas the first decay symptoms for tomatoes cultivated with far-red light started after ten days (Figure 2E, F). Red tomatoes that were prior cold stored for 10 d and cultivated at the highest far-red intensity (FR50) showed consistently lower decay values during shelf life (Figure 2G). The longest cold storage duration (15 d) resulted in no decay for FR50 tomatoes while FR0 and FR30 R tomatoes started with decay index values between 1 and 2 at the start of shelf life. These FR50 lighted R tomatoes showed the first signs of decay only after seven days (Figure 2H).

2.3.3. Tomatoes grown with additional far-red light during cultivation showed lower weight loss during shelf life

Small differences in weight loss behaviour were encountered between MG and R tomatoes during shelf life. The longer the cold storage, the higher the weight loss at the start of shelf life ($P < 0.001$), independent of the maturity stage at harvest. Lower weight loss for FR50 tomatoes was already present at day 10 for 15 d cold stored MG and R tomatoes (data not shown). In all cases tomatoes cultivated with no additional far-red lighting (FR0) showed the highest average weight loss during shelf life. In almost all cases tomatoes cultivated at the highest far-red intensity (FR50) showed the lowest average weight loss during shelf life. Differences in weight loss for tomatoes cultivated at FR0 and FR50 became larger when the cold storage duration was longer (Figure 3A-D).

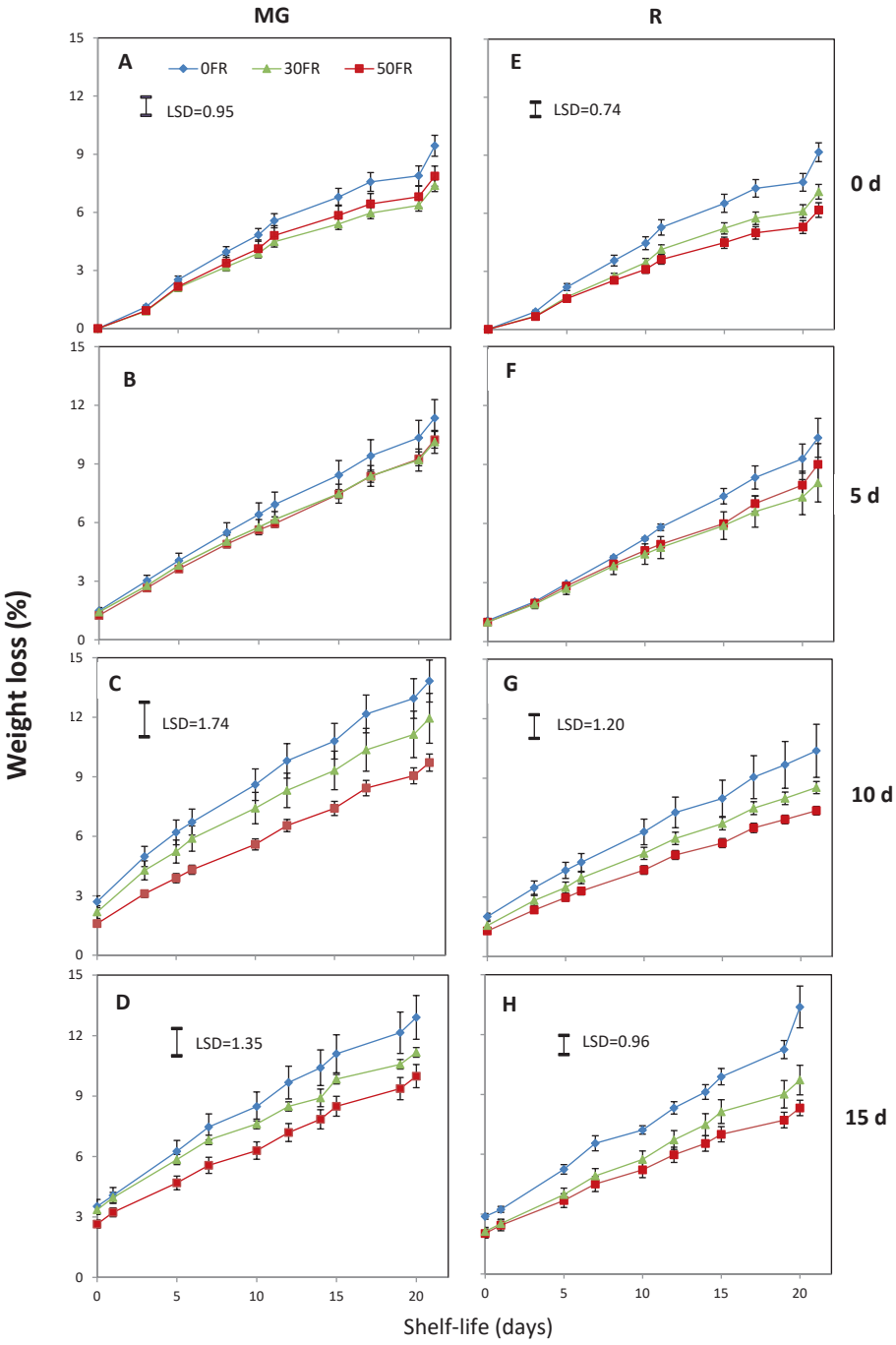

Figure 3. Weight loss of mature green (MG) and red (R) tomatoes during shelf life expressed for three light treatments: without FR (FR0) and with either 30 (FR30) or 50 µmol m^{-2} s^{-1} (FR50) additional FR light. Average weight loss with indicated standard errors is shown for 20 MG tomatoes during shelf life

at 20 °C (A) and five MG tomatoes after cold storage at 4 °C for 5 d (B), 10 d (C) or 15 d (D). The average weight loss with indicated standard error is shown for five R tomatoes during shelf life at 20 °C (E) and five R tomatoes after cold storage at 4 °C for 5 d (F), 10 d (G) or 15 d (H). LSD values ($P < 0.05$) are indicated per plot. Absence of LSD bars is indicative of no significant differences between light treatments.

2.3.4. Additional far-red light during cultivation showed faster colour development for prior cold stored mature green tomatoes during shelf life

All MG tomatoes showed normal red colouration (NAI values) and green colour loss (NDVI values) at the end of shelf life, irrespective of the light treatment and duration of cold storage (Figure 4A-D). At harvest and during cold storage no differences in NAI and NDVI values between light treatments were observed for both MG and R tomatoes (data not shown). However, red colour development was faster for MG tomatoes that were prior cold stored for 10 and 15 d cultivated at the highest FR intensity (FR50) (Figure 4C,D). No clear red and green colour change was observed for R tomatoes during shelf life, but the longer the prior cold storage, the lower the NAI values at the start of shelf life ($P < 0.001$) (Figure 4E-H). MG tomatoes showed lower NAI values at the end of shelf life when the prior cold storage period was longer (Figure 4A-D, $P < 0.001$). Longer cold storage resulted in lower NDVI values ($P < 0.001$) at the start of shelf life period for MG tomatoes (Figure 5A) and lower NAI values ($P < 0.001$) for R tomatoes (Figure 5B).

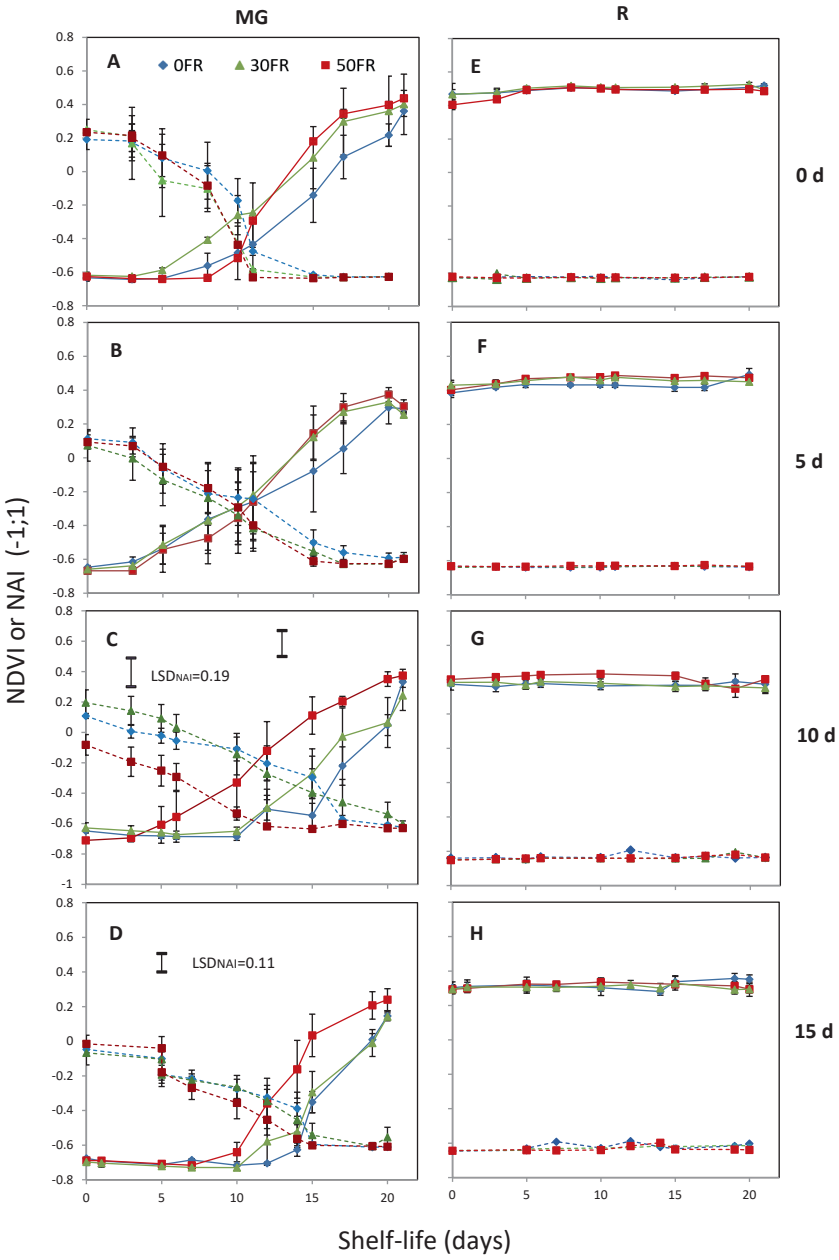

Figure 4. Colour development, expressed as average NAI values (solid lines) and NDVI values (dashed lines) with indicated standard error, during shelf life for mature green (MG) tomatoes and red (R) tomatoes for three light treatments: without FR (FR0) and with either 30 (FR30) or 50 µmol $m^{-2} s^{-1}$ (FR50) additional FR light. Colour development is shown for five MG tomatoes during shelf life at 20 °C (A) and after cold storage at 4 °C for 5 d (B), 10 d (C) or 15 d (D). The average colour development with

indicated standard errors is shown for five R tomatoes during shelf life at 20 °C (E) and after cold storage at 4 °C for 5 d (F), 10 d (G) or 15 d (H).

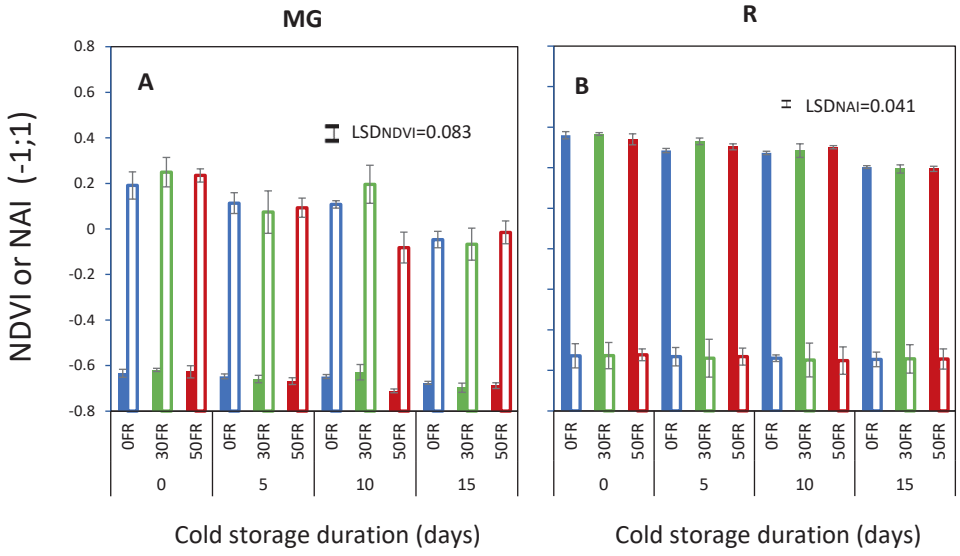

Figure 5. Average NDVI (open bars) and NAI (closed bars) values for five MG (A) and five R (B) tomatoes per cold storage duration. LSD values ($P < 0.05$) are indicated per plot. Absence of LSD bars is indicative of no significant differences between light treatments.

2.3.5. Additional far-red light during cultivation increased firmness of red tomatoes and delayed softening of MG tomatoes at harvest

Firmness at harvest (Figure 6F, $P < 0.001$) and at the start of shelf life (Figure 6G-I, $P < 0.05$) was higher with more additional FR lighting during cultivation. Firmness at harvest for MG tomatoes was unaffected by FR lighting during cultivation (Figure 6A). During cold storage no differences in firmness between light treatments were observed for both MG and R tomatoes (data not shown). However, longer cold storage decreased the firmness for both MG ($P < 0.001$) and R ($P < 0.001$) tomatoes at the start of the shelf life period, indicative of softening during cold storage (Figure 6). Mature green tomatoes cultivated at the highest FR intensity (FR50) that were not cold stored (0 d) or prior cold stored for a short period (5 d) showed higher firmness values halfway the shelf life period.

Far-red cultivation affected both tomato colour and firmness development. MG tomatoes cultivated at the highest far-red intensity (FR50) initiated colour development when fruit were firmer compared to the other light treatments. This effect was strongest for non-cold stored MG tomatoes (Figure 7A-D).

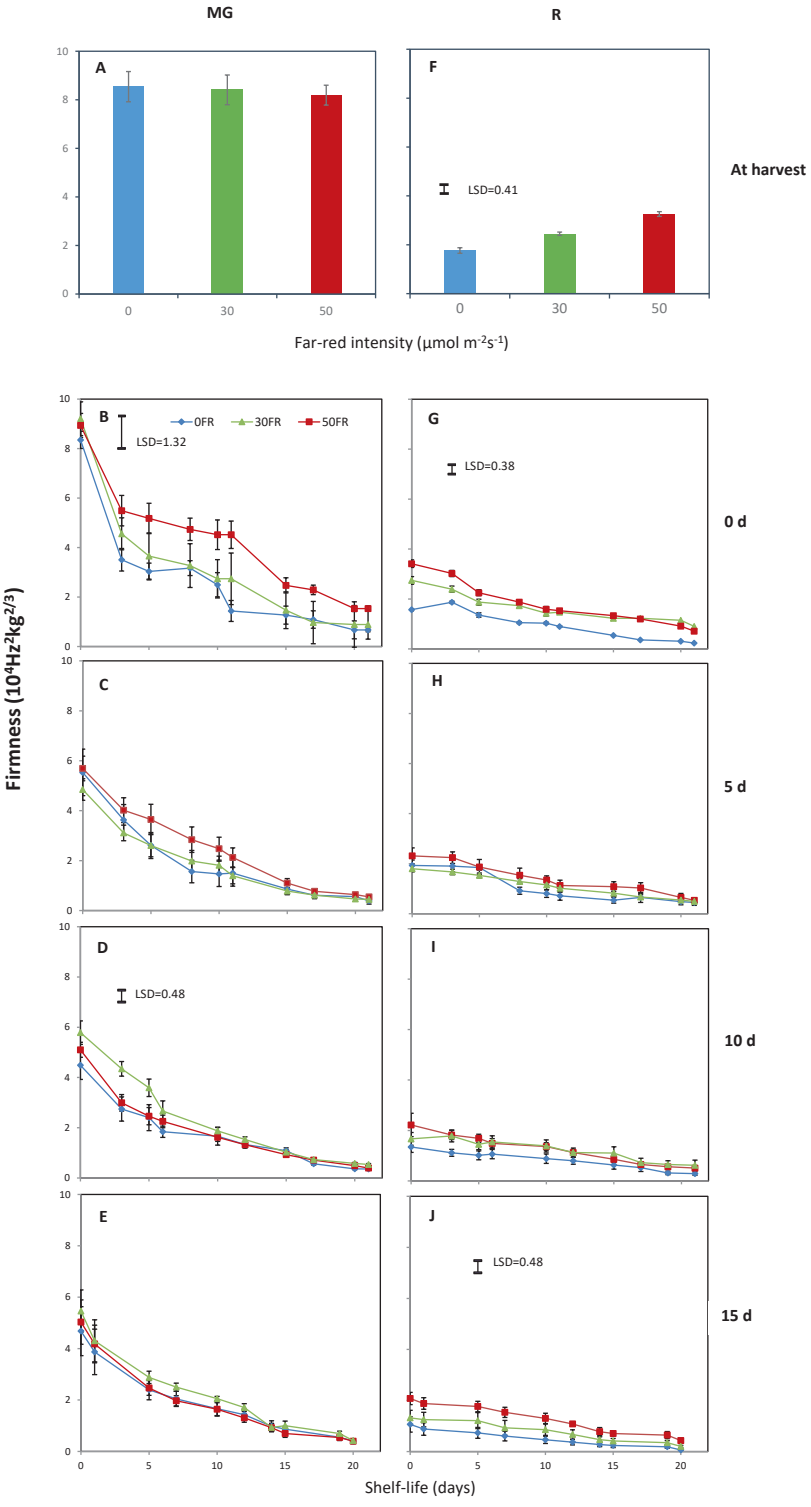
MG
R
A
F
LSD=0.41
At harvest
Far-red intensity (µmol m-2s-1)
B
0FR
30FR
50FR
LSD=1.32
G
LSD=0.38
0 d
C
H
5 d
D
LSD=0.48
I
10 d
E
J
LSD=0.48
15 d
Firmness (10⁴Hz²kg²/³)
Shelf-life (days)

Figure 6. Firmness development during shelf life for mature green (MG) tomatoes and red (R) tomatoes for three light treatments: without (FR0) and with either 30 (FR30) or 50 µmol m^{-2} s^{-1} (FR50) additional FR light. The average firmness with indicated standard error is shown for twenty MG tomatoes at harvest (A) and five MG tomatoes during shelf life at 20 °C (B) and after cold storage at 4 °C for 5 d (C), 10 d (D) or 15 d (E). The average firmness with indicated standard error is shown for twenty R tomatoes at harvest (F) and five R tomatoes during shelf life at 20 °C (G) after cold storage at 4 °C for 5 d (H), 10 d (I) or 15 d (J). LSD values ($P < 0.05$) are indicated per plot. Absence of LSD bars is indicative of no significant differences between light treatments.

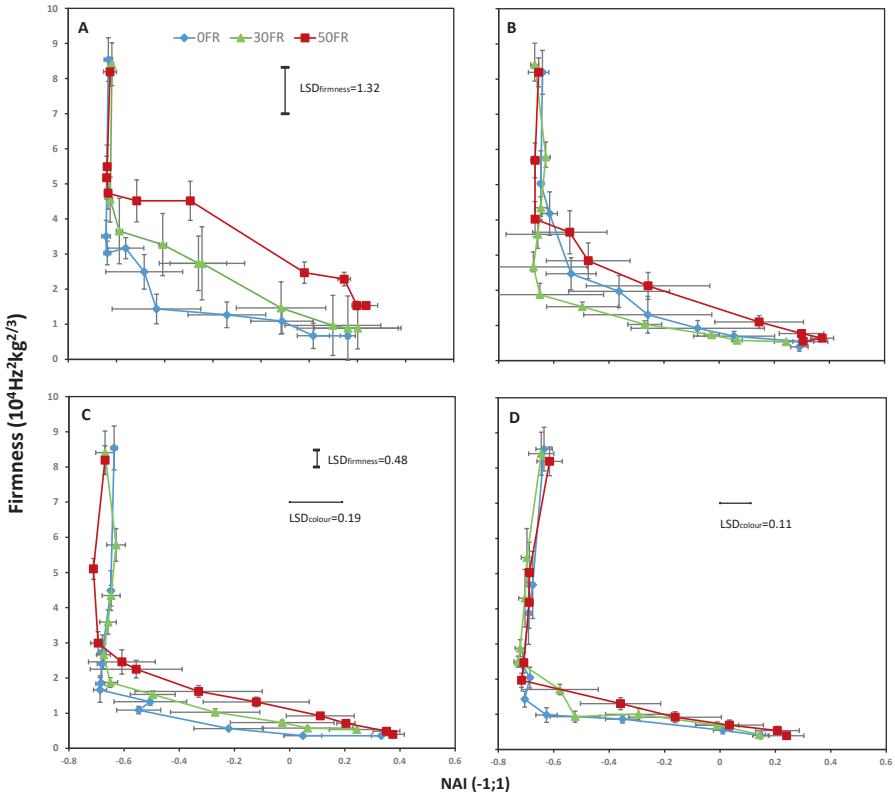

Figure 7. Firmness and colour synchronisation during shelf life for mature green (MG) tomatoes for three light treatments: without (FR0) and with either 30 (FR30) or 50 µmol m-2 s-1 (FR50) additional FR light. Average firmness and colour values with indicated standard error are shown for five MG tomatoes during shelf life at 20 °C (A) and after cold storage at 4 °C for 5 d (B), 10 d (C) or 15 d (D). LSD values (P < 0.05) are indicated per plot. Absence of LSD bars is indicative of no significant differences between light treatments.

2.4. Discussion

2.4.1. Cold storage impairs lycopene synthesis and causes lycopene breakdown in 'Moneymaker' tomatoes

Non-destructively measured NAI and NDVI values are closely related to destructively measured lycopene and chlorophyll levels in the tomato pericarp, respectively (Schouten et al., 2014). Cold storage was shown to lower NAI values and induce lycopene degradation (Farneti et al., 2012). Longer cold storage resulted in lower NAI values at the start of shelf life for R tomatoes (Figure 5B). This indicates that lycopene degradation took place during cold storage. Another indication that lycopene degradation took place is that the synchronisation between lycopene synthesis (as indicated by the NAI values) and chlorophyll degradation (NDVI values) was affected by the cold treatments as faster red colour development was not accompanied by faster chlorophyll breakdown (Figure 4B-D). Lycopene might be involved in ROS scavenging to maintain a favourable oxidative status and prevent loss of membrane integrity (Lado et al., 2015a; 2016). All MG green tomatoes developed red colour during shelf life with no blotchiness, even after 15 d of prior cold storage (Figure 4D), contrary to what was found by Biswas et al. (2014). However, longer cold storage resulted in lower NAI values reached after 20 d of shelf life for MG tomatoes (Figure 4A-D). This might indicate lycopene precursor breakdown during cold storage (Schouten et al., 2014). This would indicate that cold stored 'Moneymaker' tomatoes suffer from both lycopene precursor breakdown and lycopene breakdown, and are therefore quite sensitive to cold storage with regard to colour behaviour.

2.4.2. Additional FR light during cultivation might induce cold tolerance due to increased cuticle wax biosynthesis

Cozmuta et al. (2016) reported that red tomatoes, exposed to one month of postharvest FR lighting at 10 °C, showed higher firmness, less weight loss and lower levels of mould and yeasts due to FR induced cuticle wax biosynthesis. This resulted in a tighter cuticle-wax adhesion and enhanced barrier properties against transpiration. This effect of postharvest FR lighting on wax features might also be present in tomatoes grown with additional FR. R tomatoes were firmer at harvest if they had received additional FR lighting (Figure 4F). In addition, the decay index (Figure 4A-D) and weight loss (Figure 3A-D) during shelf life were also lower in FR treated fruit. Although firmness at harvest for MG tomatoes was not affected by FR lighting (Figure 6A), high pitting index values were observed after longer (10 and 15 d) periods of cold storage (Figure 2C, D). Remarkably, most of the pitting disappeared after 5 d of shelf life resulting in lower pitting index values for FR lighted tomatoes. Surface pitting is caused by collapse of parenchymal cells (Wang, 1990) that might be masked by increased wax biosynthesis (Cozmuta et al., 2016).

2.4.3. Cold tolerance induced by additional FR light during cultivation might benefit long tomato supply chains

Faster red colour development was observed after longer storage periods for MG tomatoes subjected to preharvest FR lighting. At the same time, less softening was found for preharvest FR lighted MG tomatoes that were either non-stored or only shortly cold stored (Figure 6A, B). This indicates that FR during cultivation affected the colour-firmness synchronization (Schouten et al., 2007; Biswas et al., 2014) of MG tomatoes during shelf life, that persisted even after 15 d of prior cold storage (Figure 7A-D). Faster red colouring due to lycopene synthesis was found for grapefruit grown inside the tree canopy (Lado et al., 2015b) which is characterised by a lower R/FR ratio (Casal, 2013). This phenomenon was also found when grapefruit where bagged during cultivation due to an accelerated chloroplast to chromoplast transition without upregulation of carotenoid genes (Lado et al., 2015b). Fruit-localized phytochromes might be involved in the colour-firmness synchronisation for MG tomatoes. Alba et al. (2000) found that light-induced accumulation of lycopene in tomato is regulated by phytochromes that do not affect pericarp softening.

FR lighting during cultivation not only increases fruit yield (Kalaitzoglou et al., 2019) but also increased cold tolerance of both MG and R tomatoes. MG tomatoes cultivated at higher FR intensities showed less pitting (Figure 2) and less weight loss (Figure 3) during shelf life. This indicates that preharvest FR lighting for MG tomatoes would benefit tomato supply chains that include long distance transport at low temperatures. For R tomatoes, FR light during cultivation resulted in higher firmness at harvest (Figure 6F), less decay (Figure 2E-H) and less weight loss (Figure 3E-H) during shelf life. This would benefit long distance transport at cold temperatures also for R tomatoes. FR lighting during cultivation might favour export as tomatoes would better withstand refrigeration during handling, transport and storage (Wang, 1994). For example, Indonesia has a tomato production surplus of about 400.000 tons (FAO, 2019), but exports only a fraction of this amount (Kementan RI, 2014) because of a lack of proper temperature management during transport (Kusumaningrum et al., 2015).

2.5. Conclusion

The aim of the research was to find out whether additional FR lighting during cultivation improves cold tolerance of R and MG tomatoes. Tomato plants received different levels of FR light during cultivation; were subsequently stored for different periods at 4 °C and thereafter chilling injury symptoms were studied during shelf life at 20 °C. Additional FR light during cultivation resulted in faster red colour development for prior long cold stored MG tomatoes, and less softening for prior short or non-cold stored MG tomatoes during shelf life. In addition, MG tomatoes showed less weight loss and less pitting during shelf life with additional FR during cultivation. For R tomatoes, additional FR light during cultivation resulted in higher firmness at harvest, less weight loss and less decay during shelf life. Taken together, these results show that FR light, applied during cultivation, induced cold tolerance in both MG and R tomatoes during cold storage. This might be caused by FR induced cuticle wax biosynthesis

with lycopene acting as antioxidant during cold storage. FR lighting during tomato cultivation might have an important role in facilitating long distance transport at low temperatures.

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CHAPTER

3

Additional blue light during cultivation induces cold tolerance in tomato fruit, but only to an optimum

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Additional blue light during cultivation induces cold tolerance in tomato fruit, but only to an optimum

Abstract

We examined the role of preharvest blue LED lighting (BL) to induce postharvest cold tolerance in 'Foundation' tomatoes. Blue and red supplemental LED light was applied to achieve either 0, 12, or 24% additional BL (0B, 12B, and 24B). Mature green (MG) or red (R) tomatoes were harvested and cold stored at 4 °C for 0, 5, 10, 15 and 20 d and thereafter stored for 20 d at 20 °C (shelf life). Chilling injury (CI) indices, colour, firmness, hydrogen peroxide, malondialdehyde, ascorbic acid and catalase activity were characterised during storage and shelf life. R fruits harvested from the 12B lighting showed increased loss of red colour during chilling and showed less CI symptoms during subsequent shelf life than R fruit from other light treatments. This indicates that R fruit from 12B acquired increased cold tolerance. MG-tomatoes showed no CI symptoms, regardless of the preharvest lighting. No effects of light treatments were found on the antioxidant capacity indicators of both tomatoes. The red colour, as measured by remittance VIS spectroscopy, is closely related to the lycopene concentration. We hypothesize that lycopene present in 12B tomatoes, compared to that of the other BL treatments, is a more efficient antioxidant, mitigating CI.

3.1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular consumer fruits often stored at low temperature to extend shelf life. Unknown to many consumers and producers, tomato is a cold-sensitive fruit that suffers from chilling injury (CI). Exposure to temperatures below 12 °C followed by storage at higher temperatures will result in reduced keeping quality, reduced flavour life and negative consumer appreciation (Biswas et al., 2012; Farneti et al., 2015). CI is caused by a sequence of events, starting with an increase in cell membrane micro viscosity, followed by increases in reactive oxygen species (ROS) causing further membrane malfunctioning (lipid peroxidation), protein oxidation, enzymatic activity inhibition and, finally, damage occurring to DNA and RNA (Sevillano et al., 2009).

Cold tolerance in tomato is mainly determined by the antioxidant capacity (Aghdam and Bodbodak, 2014). The extent of oxidative stress can be indicated by the level of malondialdehyde (MDA) which is a product of membrane lipid peroxidation (Hodges et al., 1999). Ascorbic acid (AsA), catalase (CAT) are part of the antioxidant system in tomato (Imahori et al., 2016) and are known to scavenge H_2O_2, a major ROS with a long half-life (Foyer and Noctor, 2011). One of tomato CI symptoms is lycopene degradation in red ripe tomato which does not only reduce tomato nutritional value, but also decreases its visual quality (Schouten et al., 2007). Furthermore, lycopene is considered the most efficient quencher of ROS among carotenoids (Stahl and Sies, 2003).

Recently, the role of far-red LED lighting during cultivation to induce chilling tolerance was examined. In prior long cold stored MG-tomatoes cultivated with additional far-red LED light, reduced weight loss, less pitting and faster red colour development during shelf life was observed. Red harvested tomatoes, cultivated with additional far-red, light were firmer at harvest, showed reduced weight loss and less decay during shelf life after prior cold storage (Affandi et al., 2020). Far-red LED light (non-photosynthetically active radiation) during cultivation therefore induces postharvest cold tolerance in tomato. Our aim was to investigate whether photosynthetically active radiation during cultivation is also able to induce chilling tolerance.

In strawberry, blue light (BL) illumination during postharvest storage increased the activity of antioxidant enzymes as well as the content of antioxidants such as AsA and tocopherol (Xu et al., 2014). We hypothesized that the addition of BL during tomato cultivation induces higher antioxidant capacity to protect tomato fruit against chilling induced oxidative stress. We show that supplemental BL light, up to an optimum, induced chilling tolerance in red harvested tomatoes.

3.2. Materials and methods

3.2.1. Plant material and growth conditions

Tomato (*Solanum lycopersicum* 'Foundation') seeds were sown on November 20th, 2016. Fourteen days after germination, uniform seedlings were transplanted. On February 10, 43 d after sowing, plants (34 cm tall) were transferred to the experimental glasshouse compartment of Wageningen University, the Netherlands, and treatments started. Plants were grown on rockwool slabs in a double row 'high wire' system. Climate set points were as follows: temperature 22/16 °C (day/night) and a relative humidity of 78%. The plants were irrigated with a nutrient solution (12.4 mM NO_3^-, 7.2 mM K^+, 4.1 mM Ca^{2+}, 3.3 mM SO_4^{2-}, 1.8 mM Mg^{2+}, 1.2 mM NH_4^+, 1.1 mM PO_4^{3+}, 30 μM BO_3^{3+}, 25 μM Fe^{3+}, 10 μM Mn^{2+}, 5 μM Zn^{2+}, 0.75 μM Cu^+, and 0.5 μM MoO_4^{2+}; Yara Benelux B.V., Vlaardingen, Netherlands). Electrical conductivity (2.1 dS m^{-1}) and pH (5.5) of the irrigation solution were monitored and adjusted daily. Further details of the greenhouse management are described in Kaiser et al. (2019).

3.2.2. Light treatments

Three combinations of blue and red supplemental light were obtained by combining several LED light sources, resulting in 0, 12, and 24% additional BL in a red-light background (referred to as 0B, 12B, and 24B) while keeping the total photosynthetically active radiation constant. Overhead supplemental lighting was provided by Greenpower PM-B150LO, Greenpower TL-DRBLBHO and Greenpower TLDRBMBHO modules (Philips, Eindhoven, the Netherlands) and intracanopy lighting was provided by Greenpower PMB150LO, Greenpower PM-DR150 and Greenpower interlighting DR/B modules (Philips). Once plants reached a threshold distance below overhead LED (38 cm), their stems were lowered weekly to keep their apices at a constant distance from the lamps. Overhead and intracanopy lamps were switched on 16 h before sunset, and switched off at sunset. Additionally, lamps were switched off when global radiation outside the greenhouse exceeded 450 W m^{-2}, and switched on when below 250 W m^{-2}. All side walls of the greenhouse compartment were closed off using a reflective screen, to prevent light pollution from neighbouring compartments. Light treatments were separated by white/black/white double plastic screens. Further details of the light treatments are provided in Kaiser et al. (2019).

3.2.3. Fruit selection, storage conditions and sample preparation

Sufficiently large and uniform coloured mature green (MG) and red (R) tomato fruits from all light treatments were harvested on June 19, 2017. Directly after harvest, tomatoes were randomly assigned for destructive or non-destructive analysis. The effect of the light treatments at harvest was characterised by randomly selecting 25 MG and 25 R-tomatoes per light treatment. Five randomly assigned tomatoes per light treatment and maturity stage were taken for non-destructive measurements during shelf life after dark storage for either 0, 5, 10, 15 or 20 d at 4 °C, in total 75 MG and 75 R-tomatoes. Five randomly assigned tomatoes per light treatment and maturity stage were taken for destructive analysis, four times during cold

storage. Tomatoes were dark stored for either 0, 5, 10 or 20 d at 4 °C, in total 240 MG and 240 R-tomatoes.

All tomatoes were marked on two positions on the equator for repeated colour and firmness measurements over time on the same tomatoes approximately every two days during shelf life. In addition, fresh weight and three chilling indices were assessed approximately at the same interval. Individual fruits, assigned for destructive measurements, were cut into small pieces and quickly frozen in liquid nitrogen and later ground to a fine powder for chemical analyses.

3.2.4. Colour and firmness measurements

Colour was assessed non-destructively by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany). Remittance was assessed at 570 (R570) and 780 (R780) nm by calculating the normalised anthocyanin index (NAI) (Eq. 1) which is normalized value between −1 and 1 (Schouten et al., 2014).

$$NAI = \frac{R780\text{-}R570}{R780+R570} \quad (1)$$

Non-destructive stiffness was measured using a commercial acoustic firmness tester (AFS, AWETA, Nootdorp, the Netherlands) with the tick power of the plunger set to 15. The AFS combines the single tomato resonant frequency (f in Hz) and mass (m, in kg), measured by an inbuild balance, into a FI (firmness index, Eq. 2) (Schouten et al., 2018).

$$FI = \frac{f^2 m^{2/3}}{10^4} \quad (2)$$

3.2.5. CI indices and weight loss

CI was assessed by three indices, a pitting index and uneven ripening index in MG-tomatoes, and a decay index in R-tomatoes according to Vega-García et al. (2010) with slight modifications. All indices were visually assessed by the same person with the percentage of the affected tomato surface assigned to five classes (0= no injury, 1= < 10%, 2= 11-25%, 3= 26-40%, 4= > 40%). Assessments were carried out after randomisation of the samples. Tomato weight loss was expressed as the percentage weight loss over time.

3.2.6. Total ascorbic acid measurement

AsA was measured according to the method by Davey et al. (2003) with modifications. Approximately 300 mg frozen and ground tissue per tomato was extracted with 1.5 mL ice-cold 3.3% meta-phosphoric acid (MPA) and thawed on ice. The solution was vortexed for 20 s and placed in ultrasonic bath at 0 °C for 10 min in darkness. After centrifugation (25000 *g*, 4 °C, 10 min) 1 mL extract was used for HPLC analysis of AsA. 100 µl extract was mixed with 50 µl of 5 mM dithiothreitol (DTT, in 400mM Tris base) for converting dehydroascobic acid (DHA)

into AsA. After 15 minutes incubation in darkness and room temperature, 50 µl of 8.5% o-phosphoric acid was added into the mix to stop the reaction.

The concentration of AsA was analysed using a HPLC equipped with a P580 pump (Dionex, Sunnyvale, CA, USA), a Dionex 340S UV-VIS detector and a MIDAS autosampler (Spark, Emmen, the Netherlands) equipped with a ProntoSIL 120-3 C18 AQ, 250 x 3 mm column (Knauer, Berlin, Germany). The column was eluted at a flow rate of 0.35 ml min^{-1} with 400 ul L^{-1} H_3PO4 + 2.5 ml L^{-1} MeOH + 0.1 mM EDTA in miliQ followed by a wash step with 30% acetonitrile. AsA was detected at 243 nm. The system was calibrated with a standard AsA solution (Sigma Aldrich) prepared in 3% MPA, stabilized with 2.5 mM DTT. Total AsA concentration was calculated as the sum of measured AsA and the AsA converted from DHA.

3.2.7. Catalase (CAT) measurement

Catalase activity was determined according to Nukuntornprakit et al. (2015) with a few modifications. An extraction buffer was made with 50 mM potassium phosphate at pH 7.4, 0.1 mM EDTA and 1% Triton X-100. Five hundred milligram frozen and ground tissue per tomato was homogenized with 1 mL extraction buffer at 4 °C, then centrifuged (21.100 *g*, 4 ^{0}C, 15 min) and 0.1 mL supernatant was added to 2.9 mL of reaction mixture. Reaction mixture consisted of 50 mM potassium phosphate at pH 7.0 with 15 mM H_2O_2 and 0.1 mM EDTA. The decrease in absorbance was measured for 10 min at 240 nm (25 °C) with a Varian CARY 4000 spectrophotometer (Agilent, Santa Clara, USA). One enzymatic unit (U) is defined as 0.01 absorbance decrease per minute and CAT activity expressed as U min^{-1} g fresh $weight^{-1}$.

3.2.8. Hydrogen peroxide (H_2O_2) measurement

H_2O_2 was quantified via a colorimetric method, following Junglee et al. (2014). Briefly, 300 mg sample frozen and ground tissue per tomato was extracted in 3 mL of 0.75 mL 0.1% (w/v) trichloroacetic acid (TCA), 0.75 mL 10 mM PBS (pH 7) and 1.5 mL 1 M KI. The homogenate was centrifuged (15.000 *g*, 4 ^{0}C, 15 min) and the supernatant held for 20 minutes, before obtaining the absorbance at 390 nm using a Varian CARY 4000 spectrophotometer. The absorbances were converted into H_2O_2 concentrations based on linear calibration curve.

3.2.9 Malondialdehyde (MDA) measurement

MDA was quantified via the thiobarbituric acid reactions (TBARS) method according to Zhang et al. (2016). Three hundred mg frozen tissue per tomato was homogenised with 0.9 mL 1% (w/v) TCA and centrifuged (10.000 *g*, 4 °C, 5 min). Later 250 µL supernatant was mixed with 750 µL of 0.5% thiobarbituric acid in 20% TCA+0.01% butylated hydroxytoluene. The mixture was incubated mixture at 96 °C for 30 minutes and cooled in ice for 5 minutes followed by another centrifugation (10000 *g*, 4 °C, 5 min) only to clarify suspension. Absorbance was determined at 532 nm and 600 nm for non-specific absorbance using an MDA molar extinction coefficient of 155 mmol L^{-1} cm^{-1} (Zhao et al., 2009).

3.2.10. Statistical analysis

Data measured at harvest were subjected to two-way ANOVA ($P < 0.05$) with either cold storage duration and light treatment or maturity and light treatment as factors. Data obtained during shelf life was subjected to mixed ANOVA, using SPSS 21 (SPSS, Chicago, USA) at $P < 0.05$. Mixed ANOVA was applied with light treatment and cold duration as between subject factors and shelf life days as within subject factor. Normality of the variables was tested applying the Shapiro-Wilk test. Mauchly's test of sphericity was carried out to test whether variances of the differences between all possible pairs of within-subject conditions were equal. If the sphericity assumption was not fulfilled, Greenhouse-Geisser's correction was applied to calculate the degrees of freedom. In case of a significant interactions, a pairwise comparison was carried out for each shelf life day with LSD (Least Significant Difference) values estimated.

3.3. Results

3.3.1. Effect of light treatments on CI in R and MG fruit

Increased chilling duration resulted in a higher decay index for R-tomatoes at the start of shelf life ($P < 0.001$) and overall higher decay index values during shelf life (Figure 1). Severe decay occurred in R-tomatoes after a shelf life of 20 d when prior cold-stored for 20 d. The decay index during shelf life for cold-stored R-tomatoes was consistently lower for 12B cultivated tomatoes compared to the other light treatments (Figure 1B-D). This indicates that chilling tolerance was induced for 12B cultivated R-tomatoes. In MG-tomatoes, chilling symptoms (pitting, uneven coloration) were observed only after 20 d of cold storage with no effects of light treatments (data not shown).

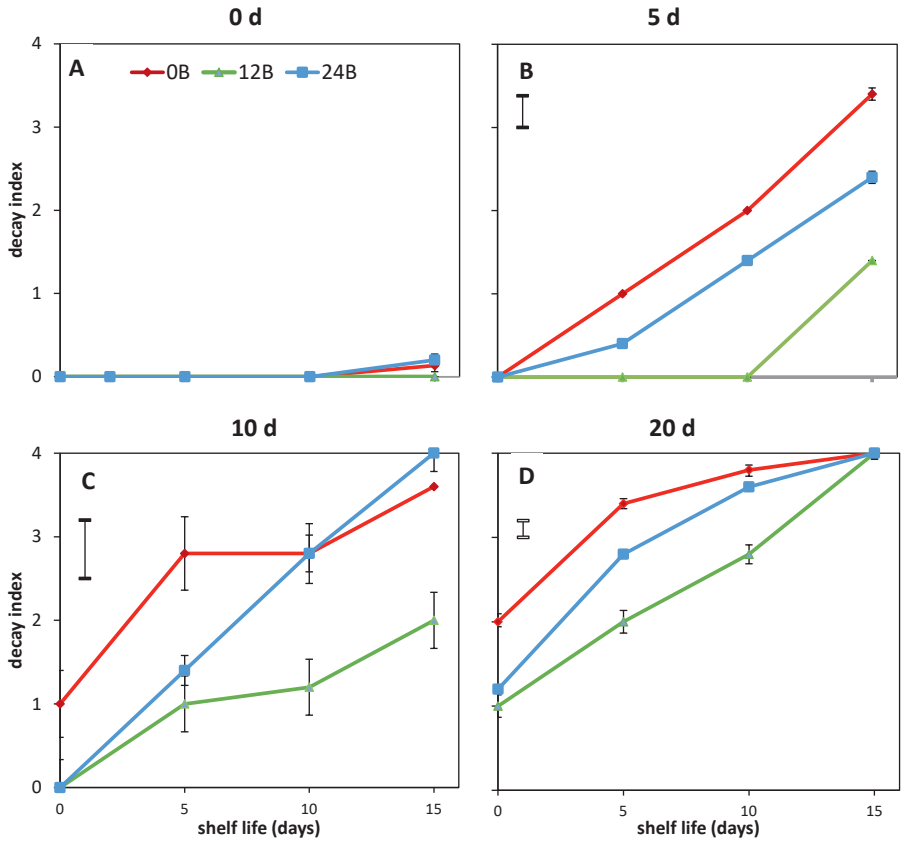

Figure 1. Average decay index with indicated standard error during shelf life (20 °C) for five red (R) tomatoes per cold storage duration. Red, green and blue symbols indicate cultivation at 0, 12 and 24% additional blue light, respectively. Tomatoes were either non-stored (A) or cold-stored at 4 °C for 5 d (B), 10 d (C) or 20 d (D). LSD values, when present, are indicated per panel.

3.3.2. Light treatments affected the colour and firmness at harvest in R-tomatoes

At harvest, R-tomatoes cultivated at 12B, showed lower NAI values compared to tomatoes cultivated at 24B (Figure2A) and higher FI index values compared to tomatoes cultivated at 0B (Figure2B). At harvest, no differences in NAI and FI values were observed for MG-tomatoes with regard to the light treatments (Figure 2).

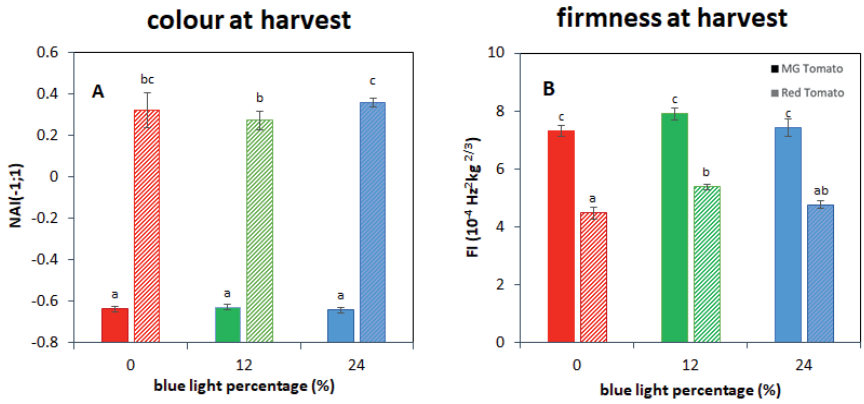

Figure 2. Average colour at harvest (A), expressed as normalised anthocyanin index (NAI) and (B) firmness, expressed as FI index, at harvest of twenty-five MG (solid bars) and R-tomatoes (dashed bars) with indicated standard error, respectively. Colours indicate tomatoes cultivated at 0 (red bars), 12 (green bars) and 24% (blue bars) additional blue light. Different letters in each panel indicate significant differences between light treatment.

3.3.3. Effect of light treatments and cold storage on coloration and softening of MG fruit

Non-chilled MG-tomatoes cultivated at 12B showed a delayed increase in NAI values (Figure 3A) compared to fruit from the other light treatments, but this effect was not observed during shelf life after cold storage (Figure 3B-D). Softening of MG-tomatoes was affected by the cold duration. Longer cold duration resulted in lower firmness at the start of the shelf life period and a lower apparent softening rate during the shelf life. Regardless of the cold storage duration, no effect of BL was observed on the softening in MG-tomatoes during storage and during shelf life (Figure 3E-H). In MG fruit, long cold storage (10 and 20 d) resulted in lower weight loss in fruits cultivated at 12B compared to fruits of the other treatments (Figure 3KL).

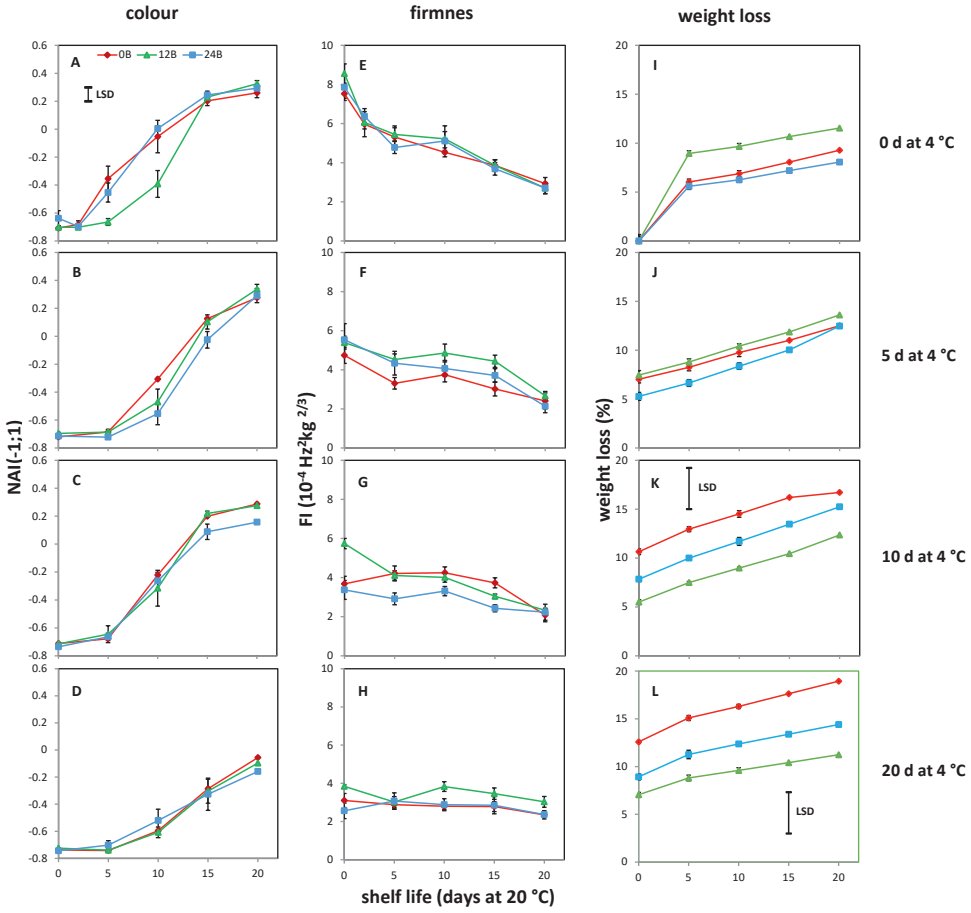

Figure 3. Average colour, firmness and weight loss development of five MG-tomatoes during shelf life at 20 °C after cold storage at 4 °C for 0 d (A,E,I) 5 d (B,F,J), 10 d (C,G,K) or 20 d (D,H,L) with indicated standard error, respectively. Colours indicate tomato cultivation at 0 (red symbols), 12 (green symbols) and 24% (blue symbols) additional blue light. LSD values, when present, are indicated per panel.

3.3.4. Cold stored R-tomatoes showed colour and firmness loss

Non chilled R-tomatoes showed constant NAI values, indicating a constant red colour during shelf life, irrespective of the light treatment (data not shown). Cold stored R-tomatoes showed lower NAI values and lower FI values the longer the duration of cold storage (Figure 4). The loss of red coloration was higher in R-tomatoes cultivated at 12B compared to the other light treatments (Figure 4A). Firmness loss during cold storage was independent of light treatments (P = 0.177, Figure 4B). Longer cold storage duration resulted in lower FI values at the start of the shelf life for R-tomatoes. No difference in softening rate during shelf life was observed for R-tomatoes when the light treatments were compared for all cold storage durations (Figure S1).

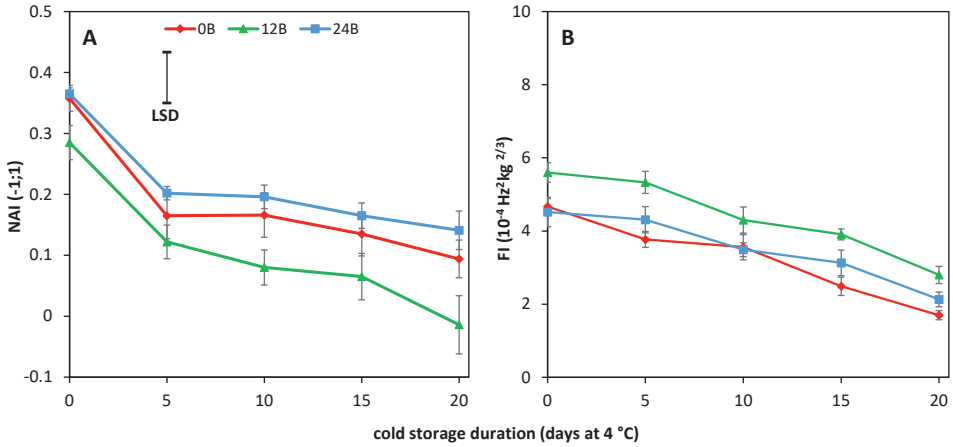

Figure 4. Average colour development, expressed as NAI values (A), and average firmness development, expressed as FI index (B), for five red tomatoes during cold storage (4 °C), with indicated standard error. Red, green and blue symbols indicate cultivation at 0, 12 and 24% additional blue light, respectively. The LSD value in panel A indicates the presence of significant differences between light treatments.

3.3.5. AsA, CAT activity, H_2O_2 and MDA content were unaffected by BL treatments

Total AsA, CAT activity, H_2O_2 and MDA contents at harvest and during cold storage were affected by the maturity at harvest. Significant differences were found between MG and R-tomatoes, with lower AsA (Figure 5A) content in MG-tomatoes compared R-tomatoes at harvest. AsA content in MG-tomatoes increased during cold storage until the same level as in R-tomatoes. Higher CAT activity (Figure 5B) but lower H_2O_2 (Figure 5C) and MDA (Figure 5D) content was observed at harvest and during cold storage for MG compared to R-tomatoes. Levels of these compounds at harvest and during cold storage were not affected by the light treatments. This indicates that the antioxidant status as indicated by these compounds is not affected by the light treatments.

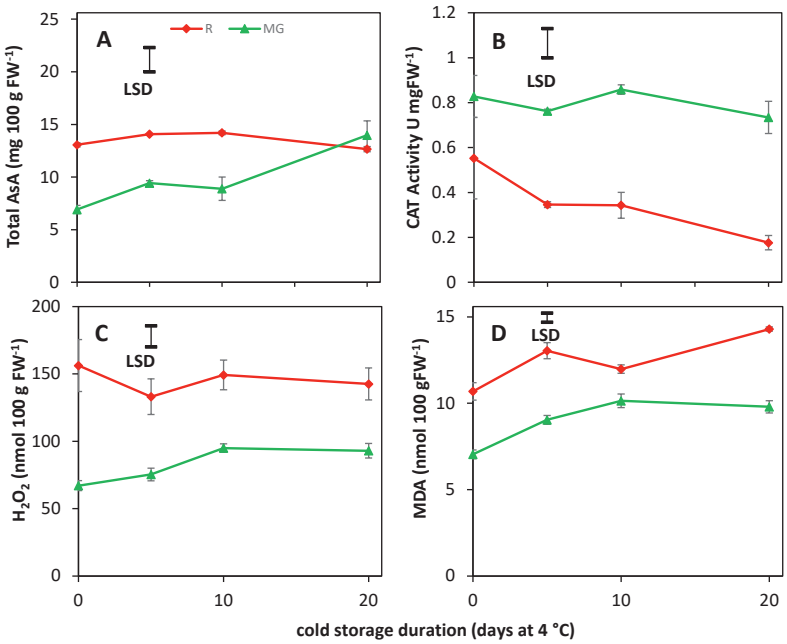

Figure 5. Changes in total AsA (A), CAT activity (B), H_2O_2 (C), and MDA (D) content at harvest and cold storage (4 °C) for fifteen MG (green symbols) and fifteen R-tomatoes (red symbols). Light treatment affects were not significant and therefore values are shown only per maturity. LSD values are indicated per panel.

3.4. Discussion

3.4.1. Cold tolerance might be related to lower lycopene levels at harvest that allow for more lycopene loss for R-tomatoes

Red tomatoes showed a higher loss of red colour when cold stored, with lower NAI values the longer the cold duration, especially for 12B cultivated tomatoes (Figure 4A). Farneti et al. (2012) and Schouten et al. (2014) showed that low temperature induced lycopene degradation in red ripe tomato could be assessed accurately by remittance spectroscopy as NAI values. There was a close relation between the NAI values and lycopene levels measured in the tomato pericarp. This might indicate that the higher cold tolerance for 12B cultivated R fruit (Figure 1) is related to a more pronounced lycopene loss during cold storage, also as no effect of light treatments were observed for AsA, CAT activity, H_2O_2 and MDA levels (Figure 5).

R-tomatoes cultivated at 12B showed higher firmness at harvest compared to 0B (Figure 2B). Higher cold tolerance of 12B R fruit (Figure 1) could be related to the higher firmness at harvest. Increased cold tolerance for R-tomatoes cultivated with additional far red was mainly linked to higher firmness at harvest that resulted in less softening during cold storage (Affandi et al., 2020). However, light treatment effects were not observed during shelf life, regardless of the cold storage duration (Figure S1). Another option is that the higher cold-tolerance for

12B cultivated fruit is linked to the lower red colour at harvest (Figure 2A). This might indicate that 12B R fruit, although having lower lycopene levels at harvest, have an increased ability to lose lycopene during cold storage (Figure 4A). The scavenging activity of lycopene is reported to be inversely correlated with its concentration (Liu et al., 2008; Kotíková et al., 2011) which suggests that more lycopene degradation provides a higher scavenging activity, perhaps due to a higher lycopene accessibility, and thus lower CI symptoms during cold storage. The cultivation of tomatoes with increased cold tolerance through increased lycopene loss during cold storage might, however, not be desirable from both a perceived quality viewpoint (Schouten et al., 2007) and a nutritional viewpoint (Salehi et al., 2019).

3.4.2. Tomato shows high variation in cold tolerance induction pathways

Lower decay values were observed for 12B R-tomatoes, but not for MG-tomatoes at any of the light treatments (Figure 1). 12B MG-tomatoes might have a small increase in chilling tolerance as lower weight loss for long stored fruits was observed (Figure 3KL) and weight loss was previously linked to chilling tolerance (Affandi et al., 2020). Nevertheless, the higher cold tolerance of R-tomatoes cultivated at 12B appears mainly late during tomato development when tomatoes were already red coloured. Analysis of the tomato plants cultivated in this BL experiment showed decreased biomass accumulation for 0B and 24B compared to 12B cultivated plants, likely caused by decreased photosynthetic light use (0B) or lower canopy light interception (24B) (Kaiser et al., 2019). It might be hypothesized that the increased chilling tolerance for 12B cultivated tomatoes is due to increased plant biomass accumulation, but this did not result in higher antioxidant capacity indicators (Figure 5) or higher fruit dry weight percentage (data not shown).

Higher cold tolerance and antioxidant capacity have been linked repeatedly. In red 'Sanibel' tomatoes, lower CAT activity, increased MDA and AsA levels were observed after cold storage for 5 d at 5 °C (Imahori et al., 2016). In the same study, cold storage for 4 d also resulted in increased H_2O_2 levels, but without CI symptoms. Induction of antioxidant related defence pathways was also shown by postharvest dips (Ding et al., 2015) or induction of heat shocks proteins (Luengwilai et al., 2012). MG-tomatoes treated with blue light during postharvest storage showed increased levels of the stress mitigator GABA and delayed colouration (Dhakal and Baek, 2014). GABA application resulted in decreased chilling injury symptoms in banana, and peach (Wang et al., 2014; Aghdam et al., 2016). This indicates multiple options to mitigate CI symptoms in tomato fruit. Our results indicate that the ability to lose lycopene or its accessibility to ROS may have increased cold tolerance. Taken together, this points to significant variation in defence pathways that are activated in response to cold storage. Investigations into gene clusters that are activated in response to cold storage might be the way forward to understand which genotypes or treatments, either applied during cultivation or after harvest, are most suitable to mitigate CI related symptoms in tomato.

3.5. Conclusion

We hypothesized that the addition of BL during tomato cultivation induces higher antioxidant capacity to protect tomato fruit against chilling induced oxidative stress. Chilling tolerance of red harvested tomato fruit was improved only by moderate blue light addition (12%) on top of a red background during cultivation. This improved cold tolerance for R fruit was not due to differences in CAT activity, total ascorbic acid, H_2O_2 and MDA levels, but due to a lower red colour at harvest and faster discolouration during cold storage. The red colour measurement, measured by remittance spectroscopy, is closely related to the lycopene concentration. It is hypothesized that the lower lycopene content of R fruit cultivated at moderate blue light levels allows for more lycopene loss during cold storage, thereby creating higher cold tolerance.

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Supplementary materials

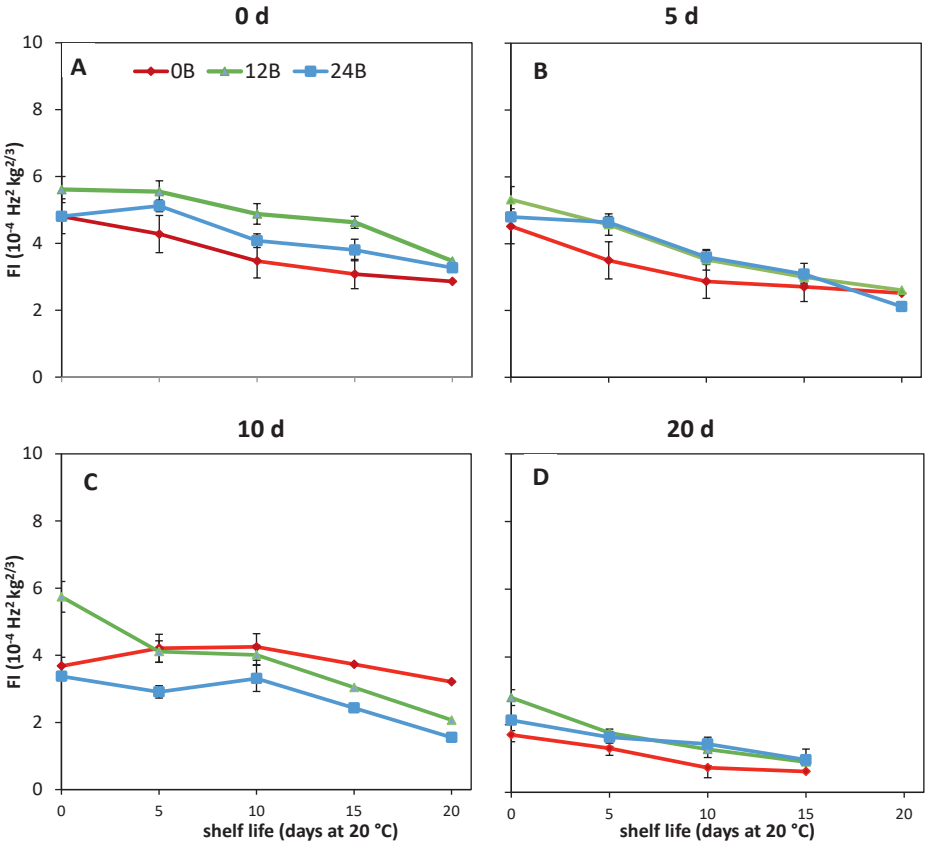

Figure S1. Average firmness index with indicated standard error during shelf life (20 °C) for five red (R) tomatoes per cold storage duration. Red, green and blue symbols indicate cultivation at 0, 12 and 24% additional blue light, respectively. Tomatoes were either non-stored (A) or cold-stored at 4 °C for 5 d (B), 10 d (C) or 20 d (D). LSD values, when present, are indicated per panel.

CHAPTER

Growth temperature affects postharvest quality properties and chilling tolerance of green harvested dwarf tomatoes

Article in preparation for submission

Additional blue light during cultivation induces cold tolerance in tomato fruit, but only to an optimum

Abstract

Effects of cultivation temperature during the phase of flowering and fruit development on tomato quality were investigated. Plants of two dwarf tomato cultivars 'Ponchi Re' and 'Tarzan', were subjected to three different growth temperatures: 16, 22 or 28 °C, starting at the flowering phase. Mature green fruit was harvested and subjected to shelf life at 20 °C for twenty days or first stored at 4 °C for fifteen days, and then placed under shelf life conditions. Fruit quality was determined through red colour development, soluble solid content (SSC), softening, weight loss, and chilling tolerance. Higher cultivation temperature increased development and production of fruit. Deviation from the 22 °C growth temperature led to increased soluble solid content in both cultivar, and smaller fruit diameter in one cultivar. Fruit grown at lower temperature had delayed colour development during shelf life, and this was further delayed by prior cold storage. 'Tarzan' showed more chilling injury (CI) symptoms than 'Ponchi Re'. In our experiment, we show that SSC can be manipulated by cultivation temperature, but that it is not associated with CI tolerance. Delayed colour formation at the lowest growth temperature demonstrated in 'Ponchi Re' tomatoes likely limited the protection lycopene offers against chilling injury (CI). For 'Tarzan' tomatoes, higher firmness at harvest, less softening and lower weight loss during cold storage in fruit from the lowest cultivation temperature likely induced increased membrane integrity, resulting in increased CI tolerance. This indicates that CI incidence depends on growth temperature and is cultivar dependent in dwarf tomato fruit.

4.1. Introduction

Tomato quality is a complex trait, governed by numerous processes at the plant and fruit level, which depend on the interplay between cultural practices, genetic, and environmental factors (Farneti et al., 2013). Tomato fruit quality is determined by colour, texture, flavour, and absence of shrivelling (Gautier et al., 2008). Those quality attributes are represented by lycopene content, firmness, volatile composition, the sugar to acid ratio, and weight loss (Bertin and Génard, 2018). Riga et al. (2008), found tomato quality properties depend more on the cumulative temperature over the course of the last 45 days before harvest than on photosynthetically active radiation (PAR). Growth temperature may also affect chilling tolerance of tomato. Chilling injury (CI) symptoms in tomato fruit are mostly visible during shelf life after prior storage below a certain, non-freezing temperature (Crisosto et al., 1999; Lurie and Crisosto, 2005; Liu et al., 2012; Biswas et al., 2016). Examples of CI symptoms are pitting, shrivelling, mealiness, uneven or delayed ripening, water-soaked areas, and higher susceptibility to bacterial and fungal rot (Ferguson et al., 1999; Lurie and Crisosto, 2005; Biswas et al., 2016). These symptoms considerably lower fruit quality and reduce its market value (Albornoz et al., 2019).

Moderate stresses such as high/low temperature, shortage or excess of water, or stresses related to the intensity and/or quality of light perceived during cultivation, trigger plants to react by initiating immediate protection against the stressor (Sabehat et al., 1998; Neta-Sharir, 2005). Exposing plants to sub-optimal growth temperatures between 10 – 20 °C during seven to ten days induced chilling tolerance in tomato plants (Barrero-Gil et al., 2016); watermelon (Lu et al., 2020) and sweet pepper (Ferguson, 1999). Acquired chilling tolerance in plants exposed to low growth temperature is contributed to expression of C-repeat-binding factors (CBF) genes (Singh et al., 2011). In addition, accumulation of small heat shock proteins (Sabehat et al., 1996, 1998; Luengwilai et al., 2012) and/or abscisic acid (Daie and Campbell, 1981; Wang and Buta, 1994; Chen and Li, 2002) may induce tolerance to low temperatures. Elevated growth temperatures, particularly close to or at harvest, may also induce tolerance to low temperatures in fruits. Cucumber fruit grown at elevated greenhouse temperatures (32 °C) showed improved tolerance to chilling compared to fruit grown at standard growing temperature, due to maintenance of higher firmness and enhanced activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Kang et al., 2001). Therefore, it seems that deviation from preferred cultivation temperatures increases CI tolerance.

Tomato has become a model crop for both quality and CI research (Campos et al., 2010). Dwarf tomato cultivars offer benefits for studying preharvest to postharvest relations due its compact size, short live cycle, and uniformity of the harvested fruit in terms of age and size (Meissner et al., 1997; Malacrida et al., 2006; Luengwilai et al., 2012; Tao et al., 2014). Mature green (MG) tomatoes are considered more sensitive to CI than red (R) tomatoes (Biswas et al., 2016). CI

symptoms in MG tomatoes may appear as complete colour inhibition after storage > 10 d (Hobson, 1981; Whitaker, 1991; Lurie and Sabehat, 1997). To test the effect of cultivation temperature on tomato quality attributes, we cultivated two dwarf tomato genotypes at three temperatures (16, 22 or 28 °C). The fruit of these plants were analysed for shelf life, with and without prior cold storage on quality properties, such as size, soluble solid content (SSC), colour, firmness, weight loss, and chilling tolerance of green harvested fruit. We show that low growth temperature induced cold tolerance in one of the dwarf tomato cultivars, but we found the opposite in the other cultivar. We discuss physiological mechanisms that might explain how growth temperature affects quality properties and chilling tolerance during cold storage and subsequent shelf life.

4.2. Material and methods

4.2.1. Greenhouse climate conditions

'Ponchi Re' and 'Tarzan' dwarf tomato plants were grown from seeds bought from Prudac (Enkhuizen, The Netherlands) in a greenhouse compartment of Wageningen University and Research in Wageningen, The Netherlands. Seeds were sown on September 11th, 2017 in a greenhouse with a photoperiod of 16 hours per day with temperature settings at 21 °C (day and night), and a relative humidity of 75%. When daylight was insufficient, additional artificial lighting (Philips 400 watt Son-T natrium lamps, Eindhoven, The Netherlands). Plants were watered manually and supported with a plastic peg for stability during fruiting. Fertiliser was applied according to commercial growth practices.

4.2.2. Climate chamber conditions and fruit harvest

When plants were fully flowering, all flowers were removed, and the temperature treatments were started. The plants were divided randomly and placed in three climate cabinets (HPS1500S, Weiss Technik, Germany) set to either 16, 22 and 28 °C. For each temperature treatment, a corresponding relative humidity was set as to ensure a constant vapor pressure difference of 0.8 kPa for all treatments (Table 1). Every week, the position of the plants within a chamber was randomised. MG tomatoes were harvested twice, one week apart. Forty five MG tomatoes per cultivar were selected in each harvest and transported within 10 minutes to the HPP lab for further storage. Per temperature/cultivar combination, six extra tomatoes were harvested for immediate destructive measurements. Tomatoes of the first harvest were stored first at 4 °C for 15 d and then placed at 20 °C for 20 days (shelf life), whereas tomatoes from the second harvest were stored immediately at shelf life condition.

Table 1. Environmental conditions, number of plants and harvested fruits per climate chamber

	16° C	22° C	28° C
Temperature (°C)	15.9 ± 0.1	22.0 ± 0.1	28.0 ± 0.1
Relative humidity (% RH)	55.2 ± 1.2	70.1 ± 0.3	79.4 ± 0.6
Number of 'Ponchi Re' plants	4	4	4
Number of 'Tarzan' plants	5	5	5
Number of harvested 'Ponchi Re' fruits	106	118	136
Number of harvested 'Tarzan' fruits	111	120	160

4.2.3. Soluble solid content

The SSC was measured using a digital refractometer (Atago refractometer PR-32α, Fukaya-Shi, Saitama, Japan). Six tomatoes were measured for each treatment. The tomatoes were cut in half. Using one half per tomato, the juice was squeezed out and used to determine the SSC (°Brix) using the refractometer. The other half of the fruit was used for dry matter content measurement. The refractometer and the knife used for cutting were cleaned with distilled water before each individual measurement. Results were expressed in ° Brix.

4.2.4. Dry matter content

The remaining halves of tomatoes from SSC measurement were weighed after cutting (fresh weight), and oven-dried at 70 °C until constant weight (24-48 hours). Fresh and dry weight (in g) were measured using a balance (Mettler PM 480, Mettler- Toledo, Leicester, UK) and subsequently expressed as the percentage fresh to dry weight.

4.2.5. Fruit diameter

The fruit size was determined by measuring the diameter (in mm) of the tomato along the equator, using a digital calliper.

4.2.6. Colour measurement

Colour was assessed non-destructively by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany). Remittance was assessed at 570 (R570) and 780 (R780) nm by calculating the normalised different vegetative index (NDVI, Eq.1) and normalised anthocyanin index (NAI, Eq. 2) which are normalized value between −1 and 1 (Affandi et al., 2020).

$$NDVI = \frac{R_{780} - R_{660}}{R_{780} + R_{660}} \quad (1)$$

$$NAI = \frac{R_{780} - R_{570}}{R_{780} + R_{570}} \quad (2)$$

The measurement head of the pigment analyser was adapted by adding a small plastic cup on top of the measurement head to collect light for colour measurements of dwarf tomatoes.

4.2.7. Firmness

Firmness was measured using a Zwick Z2.5/TS1S materials testing machine (Ulm, Germany). A probe (diameter 0.8 cm) was placed on the skin of the tomato and compressed the tomato 0.5 mm with the maximum force recorded and regarded as tomato firmness (Schouten et al., 2007). Each tomato was measured twice, and the average was taken. Measurement were taken on the equator of the fruit. After the first measurement the tomato was turned 45° for the second measurement. Results were expressed in Newton.

4.2.8. CI indices and weight loss

Each tomato was judged for the degree of CI using the chilling injury index (CII) (Vega-García et al. 2010). Three CI symptoms were measured; uneven ripening and colour development (U), pitting (P), and decay (D). The severity of each symptom is based on the percentage of affected tissue (0 = No injury 1 = < 10%, 2 = 11 – 25%, 3 = 26 – 40%, 4 = > 40%) with the CII score calculated as CII = (U+P+D)/3. Tomato weight loss over time was expressed as the percentage weight loss compared to the initial weight.

4.2.9. Data analysis

Data measured at harvest were subjected to one-way ANOVA and data obtained over the course of shelf life were subjected to mixed ANOVA, applying SPSS ver.21 (SPSS, Chicago, USA) at $P < 0.05$. Mixed ANOVA was applied with growth temperature treatment and storage temperature as between subject factors and shelf life days as within subject factor. Normality of the variables was tested applying the Shapiro-Wilk test. Mauchly's test of sphericity was carried out to test whether variances of the differences between all possible pairs of within-subject conditions were equal. If the sphericity assumption was not fulfilled, Greenhouse-Geisser's correction was applied to calculate the degrees of freedom. In case of a significant interaction, a pairwise comparison was carried out for each shelf life day with LSD (Least Significant Difference) values estimated.

4.3. Results

4.3.1. Growth temperature affected plant size and start of the fruiting period

Tomato plants were fully flowering seven weeks after sowing. At this moment, they were transferred from the greenhouse compartment to the climate cabinet. Plant morphology was affected by the growth temperature: higher growth temperature during cultivation resulted in larger and darker leaves compared to plants grown at 22 °C. Whereas plants grown at 16 °C were smaller than that of 22 °C (Supplementary Figure 1). The fruiting period was also affected; tomatoes were ready to be harvested at mature green (MG) at 12 (28 °C), 13 (22 °C) and 14 (16 °C) weeks after sowing. A higher growth temperature also correlated with increased fruit production (data not shown).

4.3.2. *Growth temperature affected soluble solid contents and diameter*

A growth temperature of 16 °C and 28 °C resulted in higher SSC (°Brix) values at harvest for both 'Ponchi Re' and 'Tarzan' tomatoes, compared with tomatoes cultivated at 22°C. (Figure 1A). No differences with respect to growth temperature were found for fruit fresh weight and fruit dry matter content (data not shown). Diameter of the MG tomatoes was not affected by growth temperature for 'Ponchi Re' tomatoes. 'Tarzan' tomatoes grown at 22 °C, had a larger diameter than those grown at both 28 °C and 16 °C (Figure 1B).

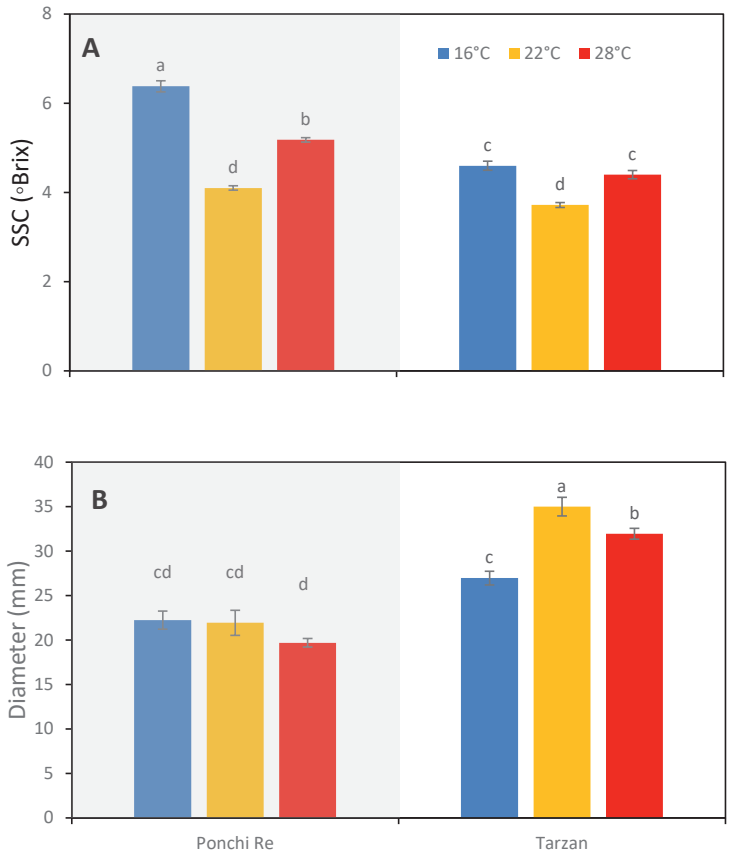

Figure 1. SSC (A) and diameter (B) of tomato grown at 16 °, 22 ° and 28 °C for 'Ponchi Re' and 'Tarzan' tomatoes at harvest. Error bars indicate standard error (n=6). Different letters indicate significant different at $P < 0.05$.

4.3.3. Low growth temperature resulted in a delay in red colour formation after harvest

After harvest all green harvested tomatoes matured to red ripe, values were measured non-destructively by the pigment analyser (Fig 2). NAI and NDVI values are representative for lycopene and chlorophyll levels in the tomato pericarp, respectively (Schouten et al., 2014). The point in time where the NAI and NDVI values cross, (cross point, CP), is an indication of the synchronisation of the chlorophyll decay and lycopene formation. In 'Ponchi Re, the CP was approximately 14, 12, and 3 d after the start of shelf life without cold storage for tomatoes cultivated at 16, 22 and 28 °C, respectively. For prior cold stored 'Ponchi Re', the CP was 17, 9, and 7 d (Figure 2A-F). For non-cold stored 'Tarzan' tomatoes the delay in colour formation due to growth temperature was less pronounced (Fig 2-G-L): 10, 8, and 5 d without storage, and 12, 7, and 9 days with prior cold storage for cultivation at 16, 22 and 28 °C, respectively. 'Ponchi Re' tomatoes showed a difference in the CP of approximately 11 d between the lowest and highest growth temperature without cold storage (Figure 2A-C). This difference is approximately only 5 d for 'Tarzan' tomatoes (Table.2). In fruit cultivated at 16 °C and 28 °C, prior cold storage resulted in a delay in the start of colour development. This delay is about 2-4 d for tomatoes of both cultivars (Figure 2). Interestingly the CP was shorter for prior cold stored compared to non-cold stored tomatoes for especially 'Ponchi Re' tomatoes (3 d) when cultivated at 22 °C (Figure 2BE).

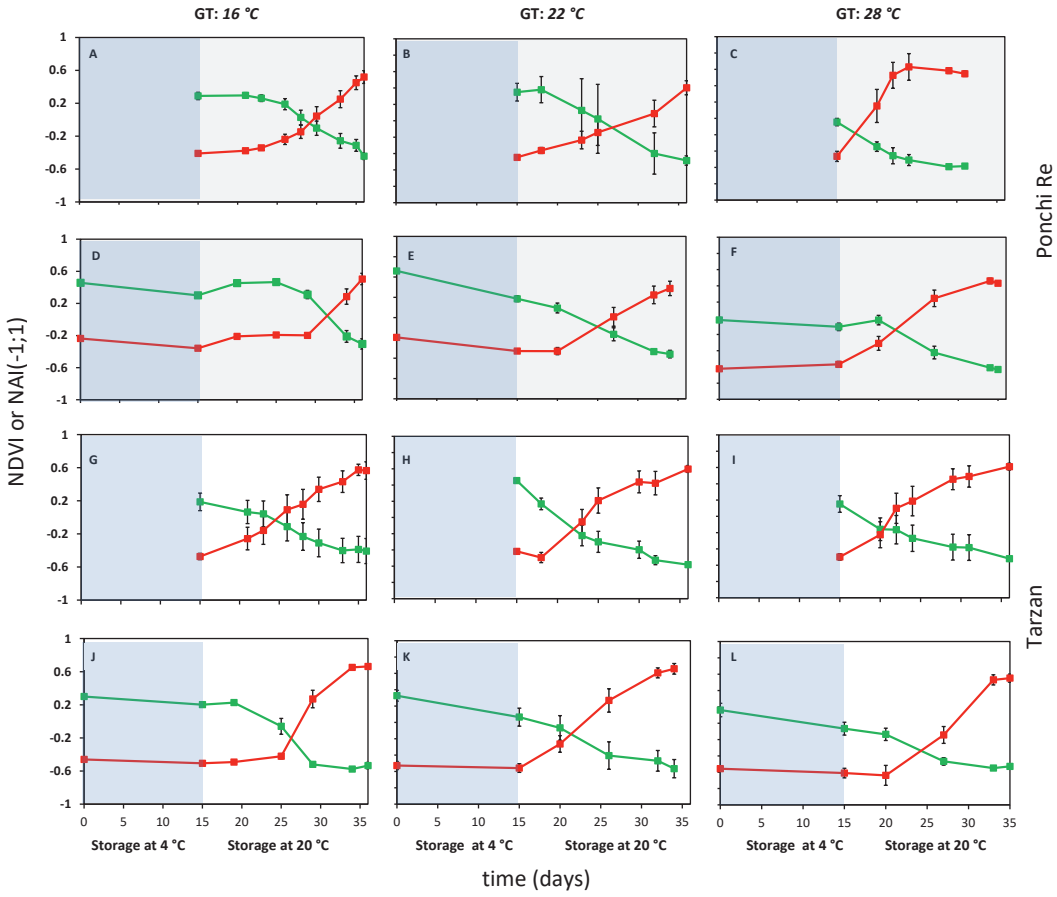

Figure 2. Colour development as indicated by NDVI (green lines) and NAI (red lines) values during cold storage at 4 °C (blue area) and during shelf life (white area) of 'Ponchi Re' (A-F) and 'Tarzan'(G-L) tomatoes cultivated at a GT (growth temperature) of 16 °, 22 ° and 28 °C. Error bars indicate standard error (n=15).

Table 2. Crossing points (CP) in days during shelf life at 20 °C of cold stored and without cold storage 'Ponchi Re' and 'Tarzan' tomatoes

		Growth temperature		
Cultivar	Storage treatment	16° C	22° C	28° C
Ponchi Re	without cold storage	14	12	3
	cold stored	17	9	7
Tarzan	without cold storage	10	8	5
	cold stored	16	6	9

4.3.4. *Growth temperature affects firmness at harvest and subsequent softening behaviour*

Firmness at harvest was lower for tomatoes cultivated at 22 °C (Figure 3AB). Cold storage resulted in less softening for tomatoes cultivated at 16 °C. (Figure 3CD). Softening during shelf life without prior cold storage was faster for 'Ponchi Re' tomatoes cultivated at 28 °C than tomatoes cultivated at 16 and 22 °C (Figure 3A). Softening for non-cold stored 'Tarzan' tomatoes was not affected by growth temperature (Figure 3B). Cold storage had no effect on the speed of softening in Ponchi Re. However, the reduced softening of Tarzan fruit grown at 16° C during cold storage was compensated by an increased softening during shelf life, to end at the same firmness as the other fruit, after 20 d of shelf life.

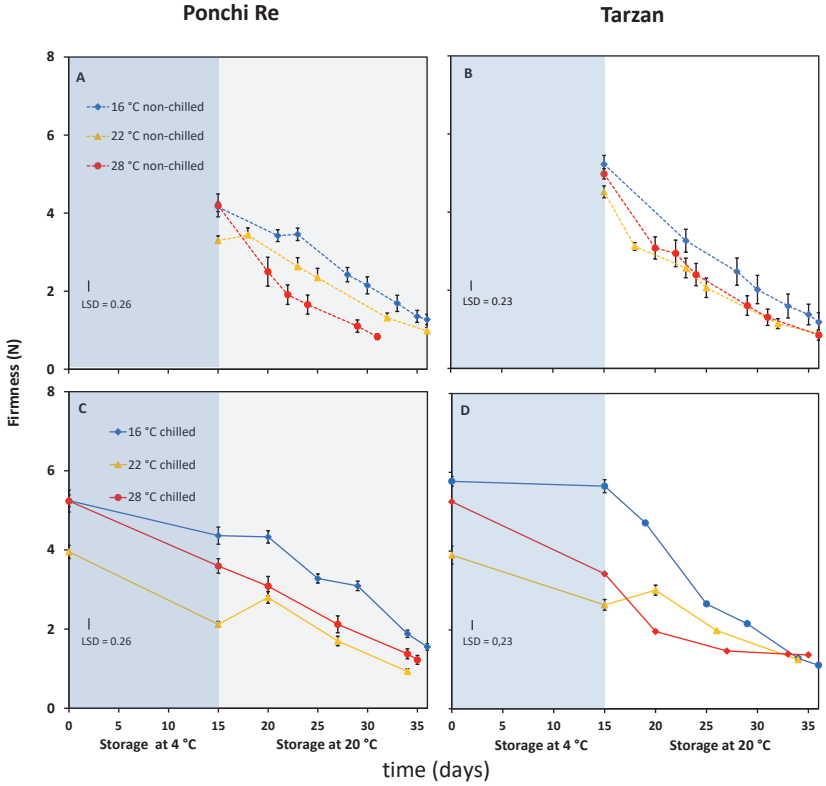

Figure 3. Firmness (N) values during cold storage at 4 °C (blue area) and during shelf life (white area) of 'Ponchi Re' (A,C) and 'Tarzan'(B,D) tomatoes cultivated at a GT (growth temperature) of 16 °, 22 ° and 28 °C. Error bars indicate standard error (n=15).

4.3.5. *Lower growth temperature reduced weight loss*

Weight loss during storage and shelf life was generally correlated with cultivation temperature. Weight loss was lowest at 16 °C, and increased for tomatoes of both cultivars cultivated at 22, and 28 °C (Figure 4). Weight loss during shelf life of prior cold stored tomatoes was similar as for non-cold stored tomatoes, with the exception of 'Ponchi Re' fruit cultivated

at 22 °C (Figure 4C). Reduced weight loss for 'Ponchi Re' grown at 16 and 28 compared with 22, could be considered a positive effect of cultivation stress on quality.

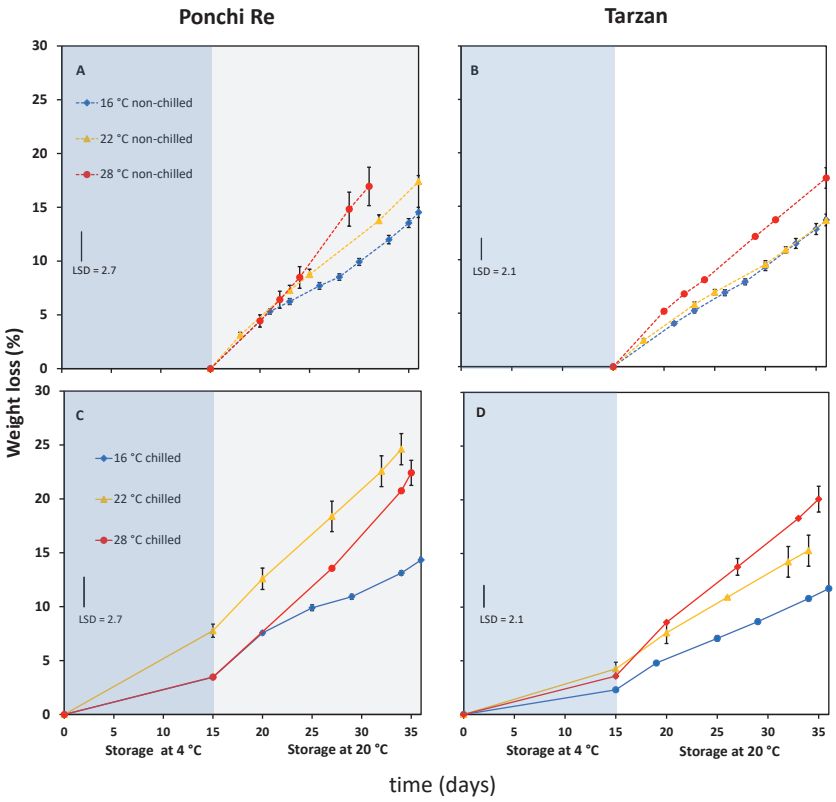

Figure 4. Weight loss percentage values during cold storage at 4 °C (blue area) and during shelf life (white area each plot) of 'Ponchi Re' (A,C) and 'Tarzan'(B,D) tomatoes cultivated at a GT (growth temperature) of 16 °, 22 ° and 28 °C. Error bars indicate standard error (n=15).

4.3.6 Growth temperature affected chilling injury incidence depending on cultivar

Chilling injury can be judged by three symptoms; uneven ripening and colour development, pitting, and decay (Affandi et al., 2020), For 'Ponchi Re' tomatoes, increasing growth temperature resulted in lower CI incidence ($P < 0.0001$) while the opposite occurred for 'Tarzan' tomatoes (Figure 5AB). 'Tarzan' tomatoes, cultivated at 28 °C, experienced higher CI index values due to higher decay and pitting. In contrast, CI index of 'Ponchi Re' cultivated at 16 °C was mainly due to higher uneven colouring and decay (data not shown).

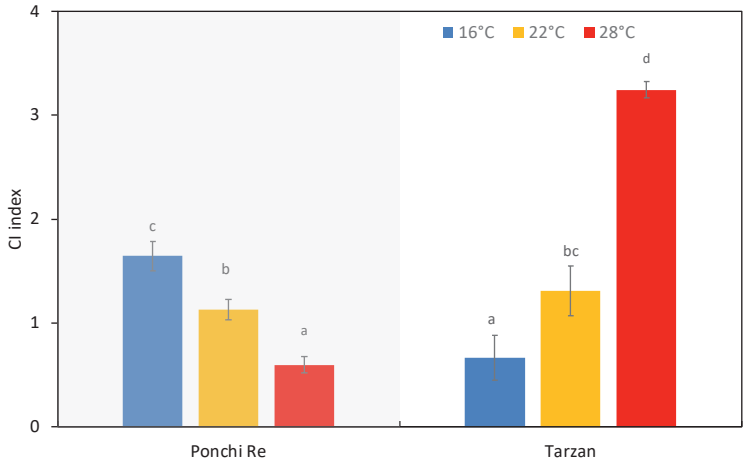

Figure 5. CI index of tomato grown at 16 °, 22 ° and 28 °C for 'Ponchi Re' and 'Tarzan' tomatoes at the end of shelf life following storage at 4 °C for 15 d. Error bars indicate standard error (n=15). Different letters in each plot indicates significant differences at $P < 0.05$.

4.4. Discussion

4.4.1. Higher SSC is not always associated with chilling tolerance

A lower (16 °C) than optimum growth temperature increased SSC of 'Ponchi Re' tomatoes (Figure 1). This is in line with the findings of Klopotek and Kläring (2014), who found increased accumulation of soluble sugars when tomato plants are exposed to growth temperature between 14–16 °C. Increased levels of soluble sugars, especially glucose, induce the ascorbate–glutathione cycle that scavenges H_2O_2 (Shao et al., 2012). Soluble sugars are also thought to act as osmoprotectant that confers freezing tolerance in plants (Ruelland et al. 2009). In addition, higher soluble sugars are associated with better cold tolerance such as in tomato (Liu et al., 2012; Zhang et al., 2019), loquat (Shao et al., 2012) and nectarines (Zhao et al., 2019). Higher SSC at harvest for 16 °C cultivated 'Ponchi Re' tomatoes was, however, associated with higher CI incidence (Figure 5). This indicates that SSC is here likely not associated with chilling tolerance. In addition, higher SCC values for 16 °C cultivated 'Ponchi Re' tomatoes softened at the same rate, indicating that this higher SSC was not related to difference in developmental stage. On the contrary, firmness at harvest was generally higher for 16 °C cultivated 'Ponchi Re' tomatoes (Figure 3). It is possible that a higher SSC may have an osmotic effect resulting in increased water containment causing higher firmness (Shackel et al., 1991; Farneti et al., 2013; Lahaye et al., 2013). 'Ponchi Re' fruits cultivated at higher than optimum (28 °C) also had higher SSC, and in this case, a lower CI index was observed. Plants cultivated at the lowest growth temperature showed smaller plants and less number of fruit and leaves

(Figure S1), it is likely that the plants grown at 16 °C were able to allocate more assimilates to fruit which caused higher SSC (Heuvelink, 1997; Beckles, 2012).

4.4.2. Low growth temperature delayed red colour development, especially for 'Ponchi Re' tomatoes

The crossing point (CP) indicates the level of synchronisation of pigment degradation and synthesis. Cold storage was reported to delay and reduce lycopene accumulation in MG tomatoes despite uninterrupted chlorophyll degradation during shelf life (Affandi et al., 2020). The CP could therefore be regarded as indicator of chilling sensitivity. 'Ponchi Re' tomatoes showed a larger difference in CP between the lowest and highest growth temperature with or without cold storage than 'Tarzan' tomatoes (Figure 2). This indicates that colour development is delayed by lower growth temperatures for especially 'Ponchi Re' tomatoes. NAI and NDVI behaviour is highly synchronised by growth temperature, with and without cold storage. This might indicate that a system that synchronises chlorophyll decay and lycopene synthesis is dependent on growth temperature. Such a system might be under the regulation SGR (STAY-GREEN) protein. SGR genes induces chlorophyll breakdown and simultaneously blocks lycopene synthesis until SGR levels decrease to a threshold that allows synthesis of lycopene to start (Hu at al., 2011; Luo et al., 2013; Sánchez-González et al., 2016). Possibly, synthesis and breakdown of SGR in 'Ponchi Re' tomatoes is more temperature dependent than in 'Tarzan' tomatoes.

Prior cold stored showed a similar delay with the non-cold stored tomatoes in the start of the colour development for both cultivars when cultivated at 16 or 28 °C (Figure 2). The opposite was shown for tomatoes cultivated at 22 °C. This means that cold storage benefitted the start of colour development for tomatoes when cultivated at 22 °C. Likely, cold storage induced the accumulation of lycopene precursors, but only when cultivated at 22° C. It is currently unclear why this happens. But it is clear that growth temperature affects colour development. For instance, Hernandez et al. (2015) showed that a high (32 °C) growth temperature decreased and later increased the lycopene content when the number of days of high temperature exposure was increased.

4.4.3. Cold tolerance might be related to membrane integrity, whereas cold sensitivity might be related to lack of ROS scavenging capacity

A lower growth temperature resulted in less softening during cold storage for both cultivars (Figure 3CD). In addition, tomatoes cultivated at 16 °C showed less weight loss in general (Figure 4). Firmness retention during chilling and shelf life is associated with higher structural cell wall integrity and reduced decay (Mirdehghan et al., 2007; Rodoni et al., 2010; Gang et al., 2015). Lower weight loss is thought to be related to higher membrane integrity leading to less decay (Saphiro and Cohen, 1994; Ali et al., 2019; Ahmed et al., 2021). Finally, a higher SSC could also lead to increased osmolarity, retaining more water resulting in higher firmness (Shackel et al., 1991; Farneti et al., 2013; Lahaye et al., 2013).

Higher firmness retention and lower weight loss in low temperature cultivated 'Tarzan' tomatoes than the control and higher temperature was associated with lower CI incidence (Figure 5). This might indicate that the cold tolerance of 'Tarzan' from low temperature cultivation is, at least partly, based on increased membrane integrity. Increased cold tolerance in terms of ability to restore development after prior exposure to low temperature (4 °C) was also found in tomato plants (Barrero-Gil et al., 2016), watermelon (Lu et al., 2020) and sweet pepper (Ferguson, 1999) when cultivated at lower temperatures. There could also be a genetic component. It is possible that 'Tarzan' may have a better regulation of the components of the chilling tolerance inducing CBF pathway (Singh et al., 2011) or produce small heat shock proteins (Luengwilai et al., 2012) when cultivated at lower cultivation temperatures.

The delay in lycopene formation in 'Ponchi Re' tomatoes cultivated at the lowest growth temperatures (Figure 2) is probably associated with the increased CI incidence at lowest growth temperature (Figure 5). Reduced lycopene formation might result in less ROS scavenging capacity as lycopene and lycopene precursors, such as phytoene and phytofluene, scavenge singlet oxygen and peroxyl radicals generated by chilling stress (Engelmann et al., 2012; Lado et al., 2016; Rey et al., 2021). This delay might trigger a cascade of oxidative processes that lead to chilling damage (Hodges et al., 2004; Martínez et al., 2014). Cold stored 'Tarzan' tomatoes cultivated at 16 °C, also experienced a delay in colour development, although to a lesser extent (Figure 2). However, this delay did not result in more CI symptoms (Figure 5). This implies that the development of CI symptoms depends not only on the ROS scavenging capacity but also on other factors, undoubtedly dependent on genotypical differences between the two cultivars used in this study, such as membrane integrity, cell wall integrity and transpiration rate of the fruit (Saphiro and Cohen, 1994; Rodoni et al., 2010; Gang et al., 2015).

4.5. Conclusion

This study assessed the effect of growth temperature during fruiting on quality properties such as SSC, colour, firmness, weight loss, and chilling injury development in two dwarf tomato cultivars. Results obtained have shown that cultivation at low temperature delayed colour development in 'Ponchi Re' and to a lesser extent in 'Tarzan' tomatoes. This delay probably contributed to the higher incidence of chilling injury symptoms in 'Ponchi Re' compared to tarzan. The lack of lycopene formation presumably results in less ROS scavenging capacity and more CI symptoms in 'Ponchi Re'. Our study also demonstrated that low growth temperature increased firmness at harvest and decreased softening during cold storage in both cultivars. This could possibly be attributed to increased membrane integrity and higher osmolarity. Because 'Tarzan' tomatoes had higher chilling tolerance when cultivated at the lowest growth temperature, it is likely that higher membrane integrity was a component

inducing higher chilling tolerance. This indicates that chilling injury incidence depends on growth temperature and genotype in the fruit of dwarf tomato plants.

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Supplementary figure 1

Figure S1. Example of temperature effect on plant morphology. All three plants shown are from the same cultivar, at 20 weeks old (fully grown). Plants were grown during fruiting in 16, 22 and 28 °C, from left to right, respectively.

CHAPTER

5

Exploring the role of low oxygen storage to reduce chilling injury in tomato

5.1. Low oxygen storage alleviates chilling injury in cherry tomatoes

Abstract

Tomato (*Solanum lycopersicum*) fruits are susceptible to chilling injury (CI) at temperatures below 12 °C and consequently, have limited possibility to benefit from low temperature storage to prolong shelf life and maintain quality. Controlled atmosphere storage likely inhibits CI by restricting oxygen availability for peroxidation of cell membrane lipids and maintaining low oxidative stress. We aimed to find oxygen concentrations that reduces CI after low temperature storage. To achieve this cherry tomatoes of two maturities (mature green, MG; and red, R) were stored at 2.5, 5 and 21 kPa O_2 combined with 0 kPa CO_2 for 14 days at 2 °C, followed by shelf life of 14 days at 20 °C. As a control, tomatoes were also stored at 12 °C under regular atmosphere. To assess the extent of chilling injury, tomatoes were evaluated for colour development, firmness behaviour and decay during shelf life. Effects of CA storage were more beneficial for MG tomatoes, showing less decay compared to non-chilled and chilled MG tomatoes stored at 21 kPa O_2. MG tomatoes stored at 5 kPa O_2 showed the best results: delayed softening (about ten days) and red coloration (about 5 days) compared to non-chilled tomatoes with no decay during shelf life. R tomatoes stored at 5 kPa O_2 showed the least decay and softening. In conclusion, chilling tolerance can be induced by CA storage at 5 kPa O_2, resulting in low decay while allowing for delayed softening but full colouration of MG tomatoes during shelf life.

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5.1.1. Introduction

Chilling injury is a set of disorders encountered by chilling sensitive fruit species during exposure to low but above freezing temperatures (Luengwilai et al., 2012; Aghdam and Bodbodak 2014). Tomatoes (*Solanum lycopersicum*) suffer chilling injury manifested as surface pitting, failure to develop colour, fast firmness loss, loss of aroma volatiles, increased susceptibility to infections with symptoms usually appearing after cold exposure at temperatures below 12 °C (Malacrida et al., 2005; Zhao et al., 2009; Imahori et al., 2016). However, storage or transportation of tomatoes at above 12 °C will promote senescence (Toor and Savage 2005). Therefore, to be able to store or transport tomatoes for longer durations at reasonably low temperature it is necessary to find alternative ways of reducing or delaying the impact of CI (Kusumaningrum, 2015).

A major cause of CI is considered to be oxidative stress (Shewfelt and Del Rosario, 2000). Low O_2 (controlled atmosphere, CA) storage limits the availability of substrate for oxidation and it also reduces oxidative respiration in the mitochondria which is a main source of electrons used to activate molecular O_2 to reactive oxygen species (ROS) (Hodges and Forney 2000). Low oxygen also reduces ethylene sensitivity of climacteric fruits (Beaudry, 2000). Low oxygen storage also increases the antioxidant pool by retaining water and lipid soluble antioxidants (Gonzalez-Aguilar et al., 2010).

CI depends on the ripening stage as levels of ROS increases throughout ripening (Hodges et al., 2004). As the fruit ripens the activity of enzymatic antioxidants increases in response to increasing ROS to maintain a balanced cellular homeostasis. Coupled with cold storage, elevated ROS levels during ripening might present an increased challenge to the fruit (Malacrida et al., 2006; Imahori et al., 2016). In tomatoes, ripening is accompanied by an increase in lycopene which is a major antioxidant, making red tomatoes more tolerant to CI (Farneti et al., 2012). Here, we explore the role of low O_2 storage and its interaction with ripening stage in reducing chilling injury in tomato.

5.1.2. Material and methods

5.1.2.1. Fruit sampling and experimental setup

Two hundred tomatoes from cherry tomato cultivar (genotype 2-003) were harvested at mature green (MG) and red (R) stage. Upon arrival the fruits were sorted on size (~2 cm diameter) and colour uniformity. Colour uniformity was assessed using a hand held pigment analyser by a green (Normalized Difference Vegetation Index (NDVI) and a red colour index (Normalised Anthocyanin Index (NAI). Green tomatoes were selected as those with NAI values between -0.50 and -0.40 and NDVI values between 0.20 and 0.45. Red tomatoes were selected having NAI values between 0.35 and 0.60 and NDVI values between -0.25 and -0.5. Fruits were left at room temperature for 24 h and then subjected to 14 days cold storage at 2 ^{0}C and 95% RH under three oxygen levels (2.5, 5 and 21 kPa) with

0 kPa carbon dioxide. As non-chilled control, tomatoes were stored at 21 kPa under regular atmosphere. Desired oxygen conditions were achieved flushing humidified gas mixtures at a flow rate of 500 mL min^{-1} with tomatoes stored in 67 L steel containers. Following cold storage, fruit were transferred to shelf life at 20 ^{0}C and 85% RH at regular atmosphere for 14 d. Repeated non-destructive measurement on colour, firmness and disorder index took place at the start of the experiment, at the last day of CA storage and during five-day intervals until day 30.

5.1.2.2. Disorder index and weight loss

Chilling injury symptoms were visually assessed with the percentage of the affected tomato surface assigned to five classes (0= no injury, 1= < 10%, 2= 11-25%, 3= 26-40%, 4= > 40%) of a disorder index. Tomato weight loss was expressed as the percentage weight loss over time.

5.1.2.3. Colour development

Colour was measured on three equatorial positions of each tomato by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) as expressed as average. Remittance was assessed at 570 (R570), 660 (R660) and 780 (R780) nm by calculating the normalised anthocyanin index (NAI) (Eq. 1) and normalised difference vegetation index (NDVI) (Eq. 2) which are both normalized value between −1 and 1 (Schouten et al., 2014).

$$NAI = \frac{R780\text{-}R570}{R780+R570} \quad (1)$$

$$NDVI = \frac{R780\text{-}R660}{R780+R660} \quad (2)$$

5.1.2.4. Firmness

Individual fruit firmness was measured at two orthogonal selected spots using a Zwick Z2.5/TS1S materials testing machine (Ulm, Germany) with a cylindrical probe (Ø 15 mm). To keep the tomatoes upright during measurement, tomatoes were placed on hard plastic ring. Firmness was determined as the maximum force needed to compress the tomato 1 mm at 50 mm min^{-1}, after lowering the probe until the tomato skin was touched (Farneti et al., 2013).

5.1.3. Results And Discussion

5.1.3.1. Low oxygen limits chilling injury incidence

In general, all chilled tomatoes showed chilling injury symptoms after 6 d in shelf life, except for MG tomatoes stored at 2.5 and 5 kPa O_2, even after shelf life of 15 d (Figure 1A). This result is consistent with findings that low O_2 reduced CI incidence in fruit (Fagundes et al., 2015; Pesis et al., 2000). The same trend also holds for R tomatoes (Figure 4.1.1B). Control MG and R fruits also showed decay, but this was related to overripening.

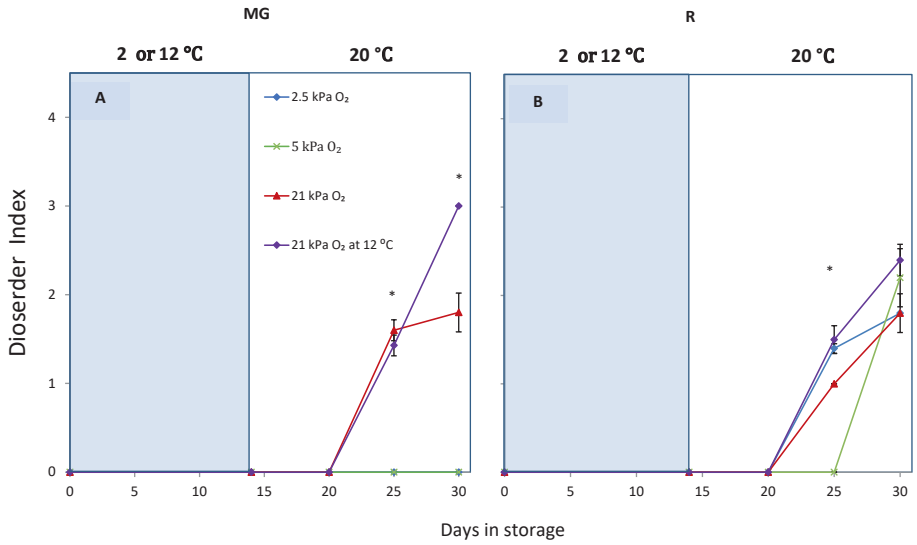

Figure 1. Chilling injury symptoms as indicated by the disorder index of MG (A) and R (B) tomatoes during cold storage at 2 or 12 °C under regular atmosphere (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The average decay index with indicated standard error is shown for five tomatoes. Stars (*) indicate significant differences between oxygen levels at given sampling time (Fisher protected LSD, $\alpha = 0.05$). Decay in MG fruit was determined by average score of pitting and uneven ripening index; decay in R fruit was determined by the average decay incidence.

5.1.3.2. Colour and firmness development

MG chilled tomatoes showed a delay in red coloration, as indicated by the increase of NAI values, of about 5 d compared to non-chilled tomatoes (Figure 2A). The chilled tomatoes stored at 2.5 and 21 kPa O_2 showed a longer delay before red colouration than the chilled tomatoes stored at 5 kPa O_2 ($P < 0.001$). The latter showed the same red colour as the non-chilled tomatoes, indicative of the ability to fully colour after chilling, perhaps even reaching higher levels when the shelf life period would have been extended. Interestingly,

Xianquan et al., 2005 found that exclusion of oxygen in storage led to better lycopene stability and reported that in the presence of oxygen during tomato processing lycopene breakdown was three times higher than in the absence of oxygen. In contrast to MG tomatoes, R tomatoes did not show any significant change in colour during and after cold storage (Figure 2B).

MG Fruit previously stored at 2.5 kPa O_2 maintained higher firmness during subsequent shelf life ($P < 0.001$). The effect of low oxygen on firmness retention is more pronounced in MG tomatoes than in R tomatoes ($P < 0.05$) with MG tomatoes stored at 5 and 21 kPa O_2 showing delayed softening of about 10 days compared to non-chilled tomatoes (Figure 3A, B). Akbudak et al.. (2009) also described that low oxygen storage result in higher firmness throughout storage.

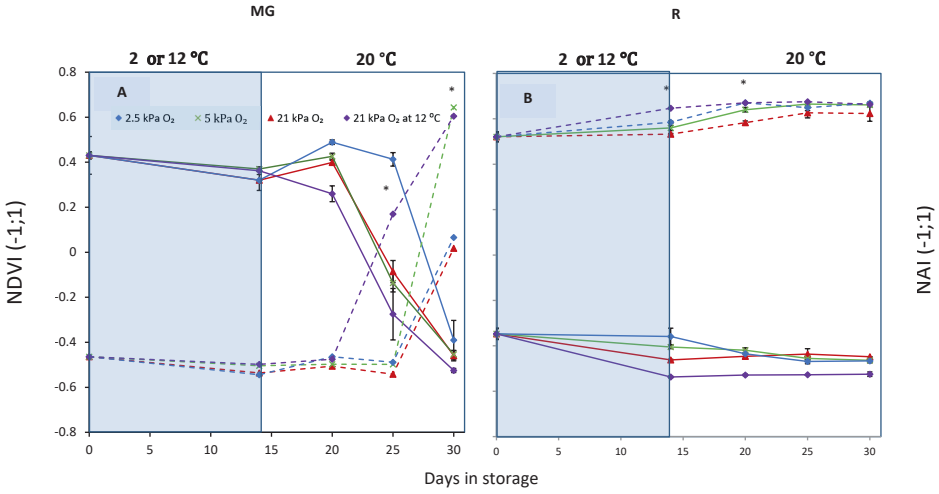

Figure 2. Colour as indicated by NDVI (dotted lines) and NAI (full lines) index of MG (A) and R (B) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The NDVI or NAI with indicated standard error is shown for five individual tomatoes (repeated measure over times). Stars (*) indicate significant differences between oxygen levels at given sampling time (Fisher protected LSD, $\alpha = 0.05$).

When colour and firmness behaviour is shown expressed against each other (Figure 4) it appears that tomatoes stored at 2.5 kPa O_2 experienced a lack of ripening both in colour and firmness development while tomatoes stored at 5 kPa O_2 only encountered a delay in firmness development. In view of the need to store tomatoes or transport tomatoes for long distances at reasonably low temperatures in especially tropical countries, the creation of MAP packaging where tomatoes are kept at 5 kPa O_2 should be explored.

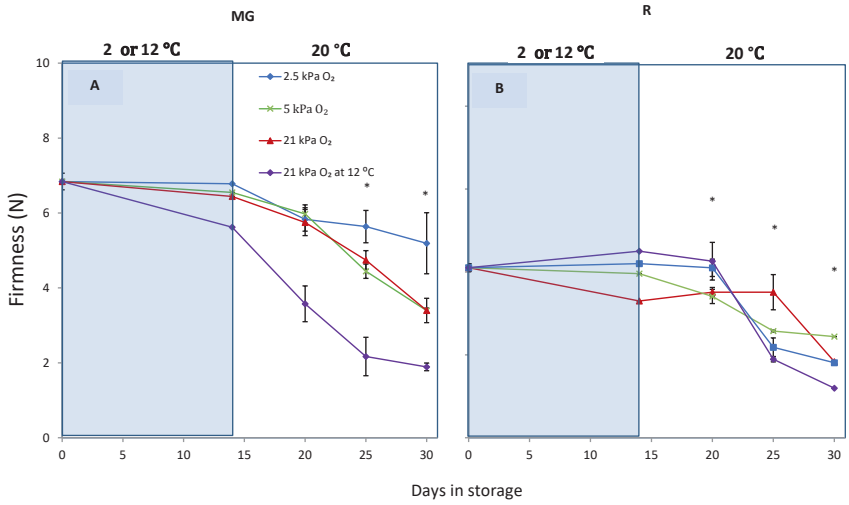

Figure 3. Firmness (N) of MG (A) and R (B) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The average firmness index with indicated standard error is shown for five tomatoes. Stars (*) indicate significant differences between oxygen levels at given sampling time (Fisher protected LSD, $\alpha = 0.05$).

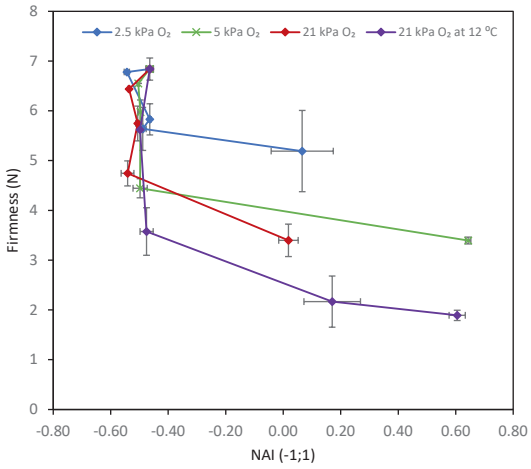

Figure 4. Firmness and colour synchronisation during cold storage and shelf life for MG and R tomatoes. Data are presented as means with indicated SE for 5 tomatoes.

5.1.4. Conclusions

Low oxygen storage (5 kPa O_2) improved chilling tolerance of cherry '2-003' tomato fruit of especially at the mature green stage through lowering decay and facilitating normal ripening.

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5.2. Low oxygen storage improves tomato postharvest cold tolerance, especially for tomatoes cultivated with far-red led light

Abstract

We investigated the effects of low oxygen storage on chilling injury development, colour development, respiration and H_2O_2 levels of 'Merlice' tomatoes cultivated with and without Far-red (FR) LED lighting during 20 days of shelf life. Mature green (MG) and red (R) tomatoes were stored at 2 °C in combination with 0.5, 2.5, 5 and 21 kPa O_2 for 15 days (experiment 1). MG tomatoes cultivated under either white LED or white LED light with FR LED light were stored at 2 °C in combination with 1, 5 and 21 O_2 kPa for 14 days (experiment 2). Chilled MG and R tomatoes from experiment 1 showed decay, firmness loss and higher weight loss during shelf life which were reduced under low oxygen conditions. FR during cultivation improved chilling tolerance of MG tomatoes. Fastest colour development and lowest respiration rate during shelf life were observed for MG fruit cultivated with FR lighting prior to storage at 1 kPa O_2/0 kPa CO_2. H_2O_2 levels during the shelf life were not affected during cold storage. The improved cold tolerance of MG tomatoes cultivated with FR lighting is likely due to lower oxygen uptake that led to both higher lycopene synthesis and less softening.

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5.2.1. Introduction

Tomato (*Solanum lycopersicum*) is a chilling sensitive fruit that will develop a disorder called chilling injury (CI) when exposed to low, but above freezing temperatures (Albornoz et al., 2019). Chilling stress disrupts metabolic processes and causes alterations in membrane fluidity, followed by an increase in reactive oxygen species (ROS) production. In addition, low enzymatic activity causes reduced ROS scavenging, which promotes development of CI symptoms (Hodges et al., 2004; Aghdam and Bodbodak, 2013; Imahori et al., 2013). CI symptoms in tomatoes include surface pitting, interrupted pigment (lycopene) synthesis, rapid softening, loss of aroma and production of off-flavours, as well as increased susceptibility to fungal infection (Maul et al., 2000; Bai et al., 2011). CI symptoms usually become visible during a shelf life period after fruits have been exposed to chilling temperatures (Maul et al., 2000; Bai et al., 2011; Zhang et al., 2013).

Controlled atmosphere (CA) storage and Modified Atmosphere Packaging (MAP) have been shown to reduce CI in mango, Japanese plum, guava, avocado and persimmon (Pesis et al., 2000; Singh, 2008; Sing and Singh, 2012; Alamar et al., 2017; de Almeida et al., 2018; Zhao et al., 2020). Low oxygen reduces respiration rate, and in addition, it may decrease ethylene production and ethylene sensitivity. CA storage downregulated the expression of Aminocyclopropane-1-carboxylic acid (ACC)-synthase and ACC-oxidase genes, responsible for ethylene synthesis (Esanhueza et al., 2014). It may also limit ROS production, which could alleviate chilling injury symptoms (Singh, 2008; Beaudry, 2000; Hodges et al., 2000). CA storage induced activation of antioxidant scavenger enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) in Japanese plum, apple and litchi (Singh, 2008; Sabban-Amin et al., 2011; Ali et al., 2016), reducing ROS, often represented by lower hydrogen peroxide (H_2O_2) levels. H_2O_2 is both a toxic metabolite and signaling molecule (Suzuki et al., 2006; Gough and Cotter, 2011). Storage under CA slowed down the activities of cell wall degrading enzymes involved in lignification and softening (Gonzalez-Aguilar et al., 2010; Mditshwa et al., 2017). In addition, low oxygen storage stabilised group VII of ethylene response factors (ERFVIIs) and transported these to the nucleus which induced expression of hypoxia-responsive genes. Hypoxia-responsive genes encode enzymes involved in sucrose catabolism (β-amylase, sucrose synthase and phosphofructokinase), fermentative metabolism (pyruvate decarboxylase, lactate dehydrogenase and alcohol dehydrogenase) and ROS scavenging (SOD, APX and CAT) (Cukrov et al., 2016; Pucciariello et al., 2017; Cukrov et al., 2019).

The severity of CI symptoms depends on the ripening stage of the fruits; mature green (MG) tomatoes are more sensitive to CI than red (R) tomatoes (Biswas et al., 2012). Comparing the responses of R and MG fruit to chilling stress is expected to provide insights into the mechanism of how low oxygen alleviates CI in sensitive tomatoes (Whitaker, 1991; Lurie and Sabehat, 1997; Biswas et al., 2012). We showed that addition of far-red (FR) lighting during cultivation alleviated CI in tomato. In MG fruit, additional FR lighting reduced weight loss,

pitting and enhanced red colour development during shelf life after prior cold storage. R fruit cultivated with additional far-red light were firmer at harvest and demonstrated reduced weight loss and less decay during shelf life after prior cold storage (Affandi et al., 2020). In the current study we investigated the effect of varying low oxygen levels on CI occurrence in mature green (MG) and red (R) tomatoes during postharvest storage. In addition, we investigated the effect of FR lighting during cultivation on CI tolerance after prior low oxygen storage.

5.2.2. Materials and methods

We carried out two experiments. In experiment 1, mature green (MG) and red (R) tomatoes were stored for 15 d at 2 °C either under regular atmosphere (21 kPa O_2, RA) or under 0.5, 2.5 and 5 kPa O_2, followed by a shelf life period of 15 d at 20 °C. In experiment 2, MG tomatoes cultivated with or without FR were harvested and stored either under RA or under 1 and 5 kPa O_2 followed by a shelf life period of 15–20 d at 20 °C. In both experiments, decay index, colour and firmness, respiration rate and hydrogen peroxide (H_2O_2) level were determined at harvest, during cold storage and during subsequent shelf life.

5.2.2.1. Plant material and growth conditions

For the first experiment, mature green (MG) and red (R) 'Merlice' tomatoes were harvested from a commercial greenhouse in Bleiswijk, the Netherlands in November 2016. The colour stage of the fruit was assessed using the NAI index (see Section 2.5). MG tomatoes were defined as tomatoes with a NAI value between −0.77 and −0.6 at harvest. R tomatoes were defined as having NAI values between 0.25 and 0.55 at harvest. For the second experiment, MG 'Merlice' tomatoes were harvested from a greenhouse at Wageningen University in May 2019 of plants grown under white LED lighting (WL) or WL with 8.3 μmol m^{-2} s^{-1} FR lighting, with a peak at 730 nm. For the FR treatment, 6% of the photons in the red region were replaced with FR. This resulted in 13 μmol m^{-2} s^{-1} FR in the FR treatment and hence this treatment was called WL + 13FR and the photon flux density was kept constant at 215 μmol m^{-2} s^{-1}. The greenhouse compartment was divided into four plots. The light intensity was 215 μmol m^{-2} s^{-1} at the top of the canopy. In this experiment, VYPRx PLUS modules (Fluence, TX, USA) were used as top lighting. For each of the plots there were six modules installed. Overhead lamps were switched on 16 h before sunset and switched off at sunset. Additionally, LED lighting was automatically switched off when the incoming sunlight exceeded 300 μmol m^{-2} s^{-1}. The spectral composition of the light treatments is shown in Figure S1. Light treatments were separated by double sided, non-transparent, white reflective plastic sheets. At harvest, uniform MG fruits were selected with a NAI value between −0.77 and −0.6. Further greenhouse management (fertigation, pollination) was conducted according to standard commercial practice.

5.2.2.2. Experimental setup

In experiment 1, MG and R tomatoes were randomly assigned into five tomatoes per maturity per CA treatment at harvest, at the end of CA storage for 15 d at 2 °C and during subsequent shelf life at 5, 10 and 15 d. This amounts to 125 MG and 125 R tomatoes. At harvest, colour and firmness was measured for all tomatoes. At each sampling point, colour, firmness and CI indices measurements were carried out. In experiment 2, the effect of far red illumination at harvest was characterised by randomly selecting 40 MG tomatoes per light treatment. Eight tomatoes per light treatment per CA treatment were assigned as a replicate of four tomatoes for repeated non-destructive measurement at harvest, after 7 and 14 d of CA storage, and after 4, 7, 10, 14 and 21 d of subsequent shelf life. Prior to sampling during at 7 d CA storage, the CA was stopped and tomatoes were taken out to be analysed. Eight randomly assigned tomatoes per light treatment and per CA treatment were taken for destructive analysis at 7 and 14 d of CA storage and after 7, 14 and 21 d of shelf life. In total 240 FR and 240 non-FR cultivated MG tomatoes were selected for this experiment.

Tomatoes were individually marked on three positions on the equator for repeated colour and firmness measurements during shelf life. In addition, fresh weight and three chilling indices were assessed approximately every 3 d during shelf life. Individual fruits, assigned for destructive measurements, were cut into small pieces and quickly frozen in liquid nitrogen and later ground into a fine powder for H_2O_2 measurements.

5.2.2.3. CA storage conditions

Tomatoes were stored at 2 °C and 95% relative humidity (RH) under low oxygen conditions followed by subsequent shelf life at 20 °C in darkness. Desired oxygen conditions were achieved by flushing humidified gas mixtures at a flow rate of 500 mL min^{-1} through 70 L stainless steel containers filled with tomatoes with an average weight of 5.15 ± 0.25 kg per container. In both experiments, tomatoes stored at RA and 2 °C served as low oxygen control whereas tomatoes stored at 12 °C and 95% RH under RA served as temperature control. All control treatment were carried out in identical containers and flow rate with the low oxygen treatments.

In experiment 1, MG and R tomatoes were subjected to low oxygen conditions of 0.5 kPa, 2.5 and 5 kPa O_2 combined with 0 kPa CO_2 (completed with balanced N_2) for 15 d. Following cold storage, fruit were transferred to shelf life conditions at 20 °C and 85% RH for 15 d. In experiment 2, MG tomatoes were subjected to low oxygen storage at 1 and 5 kPa O_2 with 0 kPa CO_2 (completed with balanced N_2). During CA storage, respiration measurements were conducted. After 14 d of cold storage, tomatoes were exposed to shelf life condition at 20 °C and 95% RH for 21 d.

5.2.2.4. Respiration measurements

In experiment 2, respiration measurements were carried out according to method previously described by our group (Schouten et al., 2007). Analysis was carried out using an Interscience Compact GC system (Interscience, Breda, NL, USA) equipped with an RT-QBond column for detecting CO_2 at the back channel and a MolSieve 5A coupled with a back pressure column type RT-QBond for the detection of O_2 at the front channel. Helium with a constant pressure of 60 and 80 kPa was used as carrier gas for the back and front channel, respectively. Each column was connected to a Thermal Conductivity Detector (TCD) set at 110 °C. CGCeditor software (v1.5.5, 2008) was used to control the setting of the CompactGC. GC was continuously connected to the samples via tubing connected to a VICI valve (model EMTMA-CE). Valve and CompactGC were coordinated by EZChrom Elite software (v3.32 SP2).

Gas measurement were conducted directly from the container. Before measurement took place, the flow through the container was stopped to allow accumulation of CO_2 and depletion of O_2 and the first GC measurement was carried out. The second measurement was carried out at the end of the incubation period. The accumulation period was approximately 5 h. The difference in gas partial pressure between the first and second GC measurements was converted into consumption and production rates according to ideal gas law methods (Bulens et al., 2011). The measurement was carried out at day 4, 6, 10 and 12 during CA storage.

5.2.2.5. Colour and firmness measurement

Colour was assessed non-destructively by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Ibbenbüren,Germany). Remittance was assessed at 570 (R570) and 780 (R780) nm by calculating the normalised different vegetative index (*NDVI*, Equation (1)) and normalised anthocyanin index (*NAI*, Equation (2)) which are normalised value between −1 and 1 (Schouten et al., 2014).

$$NDVI = \frac{R_{780} - R_{660}}{R_{780} + R_{660}} \quad (1)$$

$$NAI = \frac{R_{780} - R_{570}}{R_{780} + R_{570}} \quad (2)$$

Firmness was measured non-destructively using a commercial acoustic firmness tester (AFS, AWETA, Nootdorp, the Netherlands) with the tick power of the plunger set to 15. The AFS combines the single tomato resonant frequency (f in Hz) and mass (m, in kg), measured by an inbuild balance, into a *FI* (firmness index) (Schouten et al., 2018) (Equation (3)).

$$FI = \frac{f^2 m^{2/3}}{10^4} \quad (3)$$

5.2.2.6. Disorder index and weight loss

CI was assessed by three indices, a pitting index and uneven ripening for MG fruit, and a decay index for R tomatoes according to the previously described method (Affandi et al., 2020).

All indices were visually assessed with the percentage of the tomato surface assigned to five classes (0 = no injury, 1 = < 10%, 2= 11–25%, 3= 26–40%, 4 = > 40% affected area). The average score of pitting and uneven ripening index for MG, and decay index tomatoes were termed general disorder index. Tomato weight loss over time was expressed as the percentage weight loss (*WL*, in%) with W_0 the initial weight (in g) and W_t the weight (in g) according to Equation (4).

$$WL = \frac{W_0 - W_t}{W_0} \times 100 \quad (4)$$

5.2.2.7. Hydrogen peroxide (H_2O_2) measurement

H_2O_2 was quantified via a colorimetric method (Junglee et al., 2014). Briefly, a 300 mg sample of frozen and ground tissue per tomato was extracted in a solution containing of 0.75 mL 0.1% (*w*/*v*) trichloroacetic acid (TCA), 0.75 mL 10 mM phosphate buffer (pH 7) and 1.5 mL 1 M KI. The homogenate was centrifuged (15,000× *g*, 4 °C, 15 min) and the supernatant transferred to a new tube and allowed to sit at RT for 20 min before obtaining the absorbance at 390 nm using a Varian CARY 4000 spectrophotometer (Agilent, Santa Clara, CA, USA). Measured absorbances were converted into H_2O_2 concentrations using a calibration curve constructed with a commercial O_2 solution (Sigma Aldrich, St. Louis, MI, USA).

5.2.2.8. Statistical analysis

Data obtained during shelf life were subjected to mixed ANOVA, applying SPSS ver.21 (SPSS, Chicago, IL, USA) at $P < 0.05$. Data from the first experiment were analysed by mixed ANOVA with oxygen level and maturity as between subject factors and days in storage as within subject factor. For the second experiment, mixed ANOVA was carried out with oxygen level and FR as between subject factor and days in storage as within subject factor. Normality of the variables was tested applying the Shapiro-Wilk test. Mauchly's test of sphericity was carried out to test whether variances of the differences between all possible pairs of within-subject conditions were equal. If the sphericity assumption was not fulfilled, Greenhouse-Geisser's correction was applied to calculate the degrees of freedom. In case of a significant interaction, a pairwise comparison was carried out for each shelf life day with LSD (Least Significant Difference) values estimated.

5.2.3. Results

5.2.3.1. Experiment 1: effects of low oxygen conditions on CI indices, weight- and firmness loss

In the first experiment, typical CI symptoms such as pitting, uneven colouring and decay were observed for both MG and R tomatoes during low oxygen storage and shelf life. Storage at 0.5 kPa oxygen resulted in necrosis, fungal infection and rotting and were therefore omitted from this study. In MG tomatoes there were generally no visible CI symptoms observed during cold storage, except for tomatoes stored at 5 kPa O_2 (Figure 1A). During the shelf life, fruit (MG and R), prior stored at 2.5 kPa O_2, showed the lowest, and RA the highest disorder (Figure 1). MG

tomatoes from the temperature control (12 °C) also developed some pitting, comparable to the tomatoes stored at 2.5 kPa O_2. R tomatoes stored at 12 °C (temperature control) developed the least decay. At 2 °C, the R tomatoes stored at 2.5 kPa showed the least decay while the fruit stored at RA developed the highest disorder after 20 d of shelf life which prevented further measurements. On the other hand, R tomatoes from the temperature control (21 kPa at 12 °C) developed the lowest decay ($P < 0.0001$). This indicated that the storage at 12 °C also resulted in a small amount of CI symptoms. In general, MG tomatoes developed slower pitting than R tomatoes, indicating that R tomatoes were surprisingly more sensitive to cold storage than MG tomatoes.

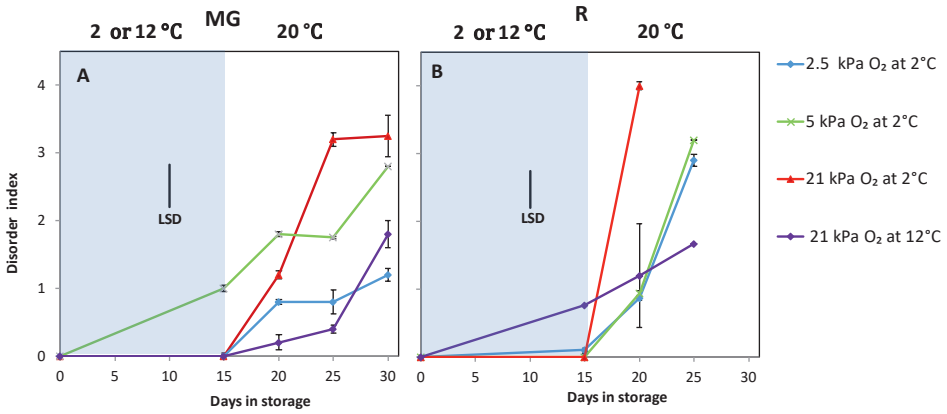

Figure 1. Chilling injury symptoms as indicated by the disorder index of MG (**A**) and R (**B**) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The average decay index with indicated standard error is shown for five tomatoes. LSD values ($P < 0.05$) are indicated per panel. Disorder in MG fruit was determined by averaging the values of the pitting and uneven ripening index; disorder in R fruit was determined by the average decay incidence.

Weight loss was higher for MG compared to R tomatoes (Figure 2). Fruit stored at 12 °C showed highest weight loss. The lowest weight loss for both MG and R tomatoes was observed in fruit that had been stored at 2 °C and 2.5 kPa O_2 ($P< 0.005$). Fruit stored at 12 °C and stored at 2.5 or 5 kPa O_2 at 2 °C showed less softening compared to fruit stored at 2 °C and 21 kPa O_2 (Figure 3).

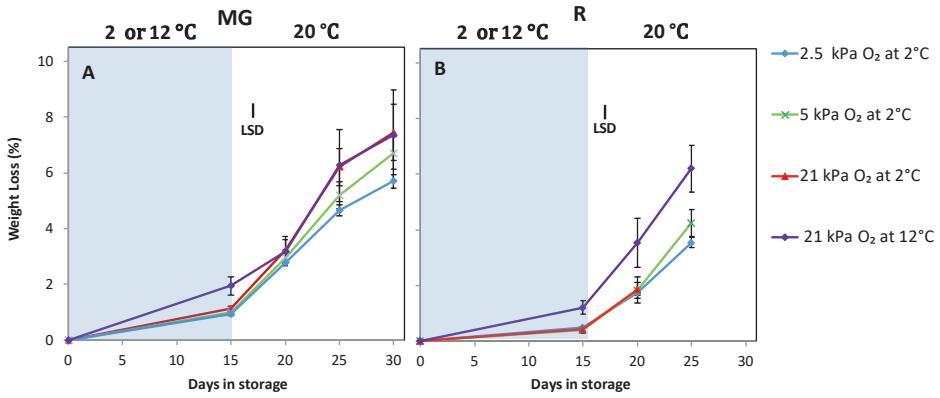

Figure 2. Weight loss of MG (**A**) and R (**B**) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The average weight loss with indicated standard error is shown for five tomatoes. LSD values ($P < 0.05$) are indicated per panel.

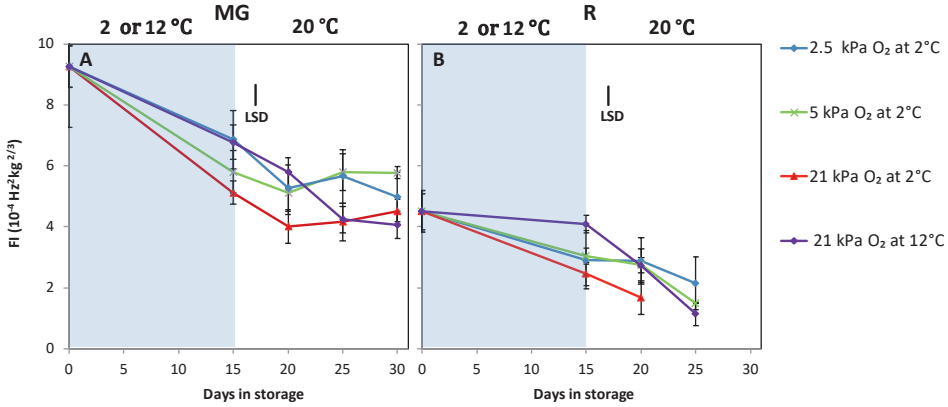

Figure 3. Firmness index of MG (**A**) and R (**B**) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The average firmness index with indicated standard error is shown for five tomatoes. LSD values ($P < 0.05$) are indicated per panel.

5.2.3.2. Experiment 1: effects of low oxygen conditions on tomato colour development

Red colour formation for MG fruit, as indicated by NAI values, was limited for all fruit that had been stored at 2 °C, independent of the oxygen level. Fruit stored at 12 °C showed colouration during subsequent shelf life at 20 °C. (Figure 4A). During low oxygen storage, more chlorophyll breakdown was observed with increasing oxygen levels. In R tomatoes, all treatments, except for the tomatoes in the temperature control, showed a reduction in the NAI

values during cold storage. During shelf life, fruit from all treatments showed increasing NAI values, except for the RA control (Figure 4B).

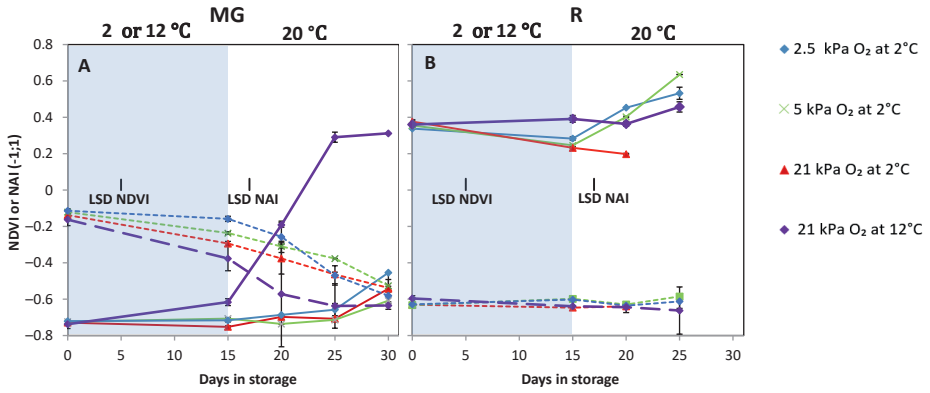

Figure 4. Colour as indicated by NDVI (dotted lines) and NAI (full lines) index of MG (**A**) and R (**B**) tomatoes during cold storage at 2 or 12 °C (blue area) and subsequent shelf life at 20 °C (white area). Blue, green, red and purple lines and symbols indicate 2.5, 5, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C and 21 kPa O_2 at 12 °C (temperature control), respectively. The NDVI or NAI with indicated standard error is shown for five individual tomatoes (repeated measure over times). LSD values ($P < 0.05$) are indicated per panel.

5.2.3.3. Experiment 2: effects of low oxygen storage of mature green tomatoes cultivated with and without FR lighting on CI symptoms, weight- and firmness loss

Tomatoes cultivated without far red lighting during cultivation showed CI symptoms during shelf life. The lowest pitting index was observed for MG tomatoes stored at 1 kPa O_2, the highest for the low oxygen control (Figure 5A). MG tomatoes cultivated with far-red lighting demonstrated reduced CI compared with tomatoes grown without FR lighting. In fact, no CI symptoms were observed for all low oxygen treatments, even after 3 weeks of shelf life (Figure 5B). There were no chilling symptoms in fruit stored at 12 °C, and no differences were observed in terms of weight loss (Figure S2).

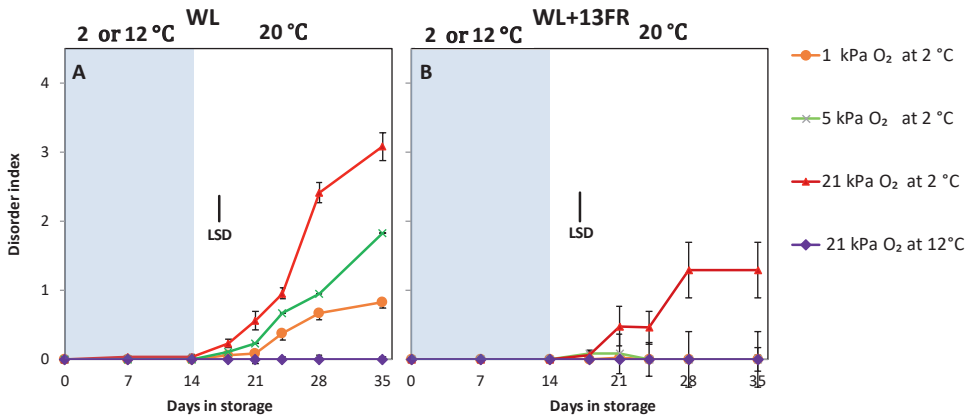

Figure 5. Chilling injury symptoms as indicated by the disorder index of MG tomatoes cultivated under white LED light (WL) without far red lighting (**A**) or with far red lighting during cultivation (**B**) during storage (blue area) at 2 or 12 °C and shelf life at 20 °C (white area). Orange, green, red and purple lines and symbols indicate 1, 5 and 21 kPa O_2 (low oxygen control) applied during storage at 2 °C, and 21 kPa O_2 at 12 °C (temperature control), respectively. The average disorder index with indicated standard error is shown for two replicates of four tomatoes ($n = 2$); (repeated measure over times). LSD values ($P < 0.05$) are indicated per panel.

Firmness at harvest was similar for MG tomatoes cultivated with or without FR lighting ($P > 0.05$). Softening during storage at 2 °C for was faster for MG tomatoes that were cultivated without- compared to those with FR lighting ($P < 0.05$) (Figure 6). Softening of tomatoes cultivated without FR was similar during storage and shelf life independent of the storage oxygen concentration. Tomatoes cultivated without FR from the temperature control treatment showed no softening during storage (Figure 6A), but tomatoes cultivated with FR showed similar softening for all treatments (Figure 6B).

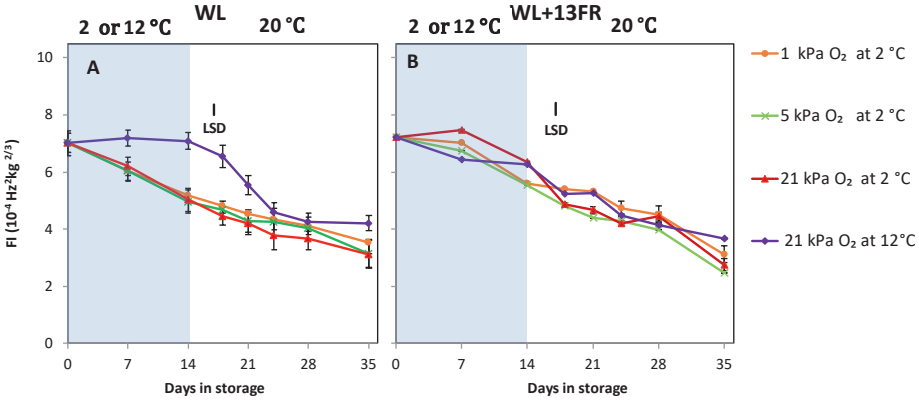

Figure 6. Firmness as indicated by firmness index (FI) of MG tomatoes cultivated under white LED light (WL) without far red lighting (**A**) or with far red lighting during cultivation (**B**) during cold storage (blue area) at 2 or 12 °C and subsequent shelf life at 20 °C (white area). Orange, green, red and purple

lines and symbols indicate 1, 5 and 21 kPa O_2 (low oxygen control) applied during storage at 2 °C, and 21 kPa O_2 at 12 °C (temperature control), respectively. The average firmness index with indicated standard error is shown for two replicates of four tomatoes ($n = 2$); (repeated measure over times). LSD values ($P < 0.05$) are indicated per panel.

5.2.3.4. Effects of low oxygen conditions on colour development of mature green tomatoes cultivated with and without FR lighting

During cold storage red colour development was blocked, as indicated by the constant NAI values, irrespective of low oxygen treatments for both MG fruit cultivated with and without FR lighting. Colour development for the temperature control tomatoes started immediately, although faster for the MG tomatoes cultivated with FR lighting (Figure 7). During shelf life, colour development was similar for the different low temperature oxygen storage treatments in fruit without FR lighting. Fruit cultivated with FR lighting reached higher NAI values in fruit prior stored at the low oxygen concentrations ($P < 0.001$) (Figure 7B). NDVI values were not significantly affected by oxygen level nor FR treatment.

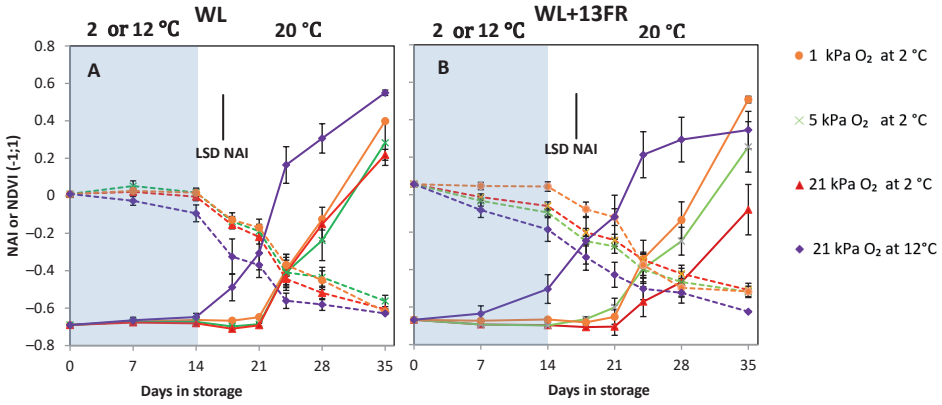

Figure 7. Colour indicated by NDVI (dotted lines) or NAI (full lines) values of MG tomatoes cultivated under white LED light (WL) without far red lighting (**A**) or with far red lighting during cultivation (**B**) during cold storage (blue area) at 2 or 12 °C and subsequent shelf life at 20 °C (white area). Orange, green, red and purple lines and symbols indicate 1, 5 and 21 kPa O_2 (low oxygen control) applied during storage at 2 °C, and 21 kPa O_2 at 12 °C (temperature control), respectively. The average NDVI and NAI values with indicated standard error are shown for two replicates of four tomatoes ($n = 2$). LSD values ($P < 0.05$) are indicated per panel.

5.2.3.5. Effects of low oxygen conditions on respiration and H_2O_2 production of mature green tomatoes cultivated with and without FR lighting

Respiration rate measurements were carried out from the fourth day onwards to allow time to achieve the set low oxygen conditions. The O_2 consumption rate at 2 °C was observed to be lower for MG tomatoes stored at lower oxygen levels (Figure 8A,B). At 12 °C, both CO_2 production and O_2 consumption was higher than at 2 °C. The CO_2 production rate, however,

was similar at the low oxygen levels (Figure 8C,D). The oxygen consumption rate over time was lower for MG fruits cultivated with FR lighting and stored at 1 kPa O_2.

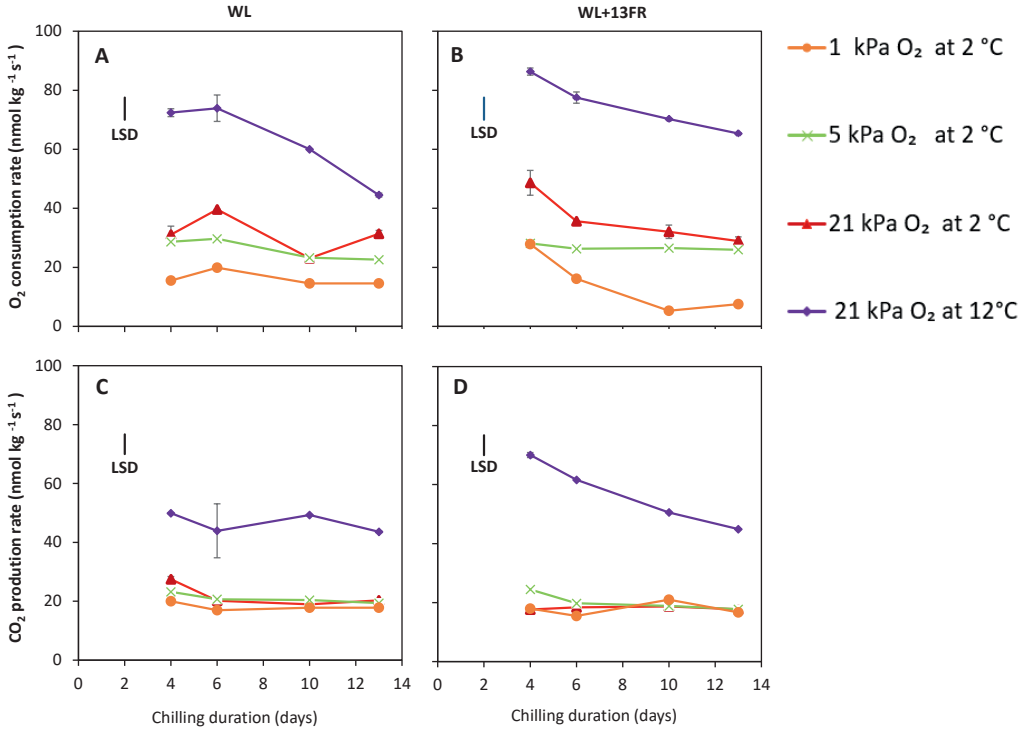

Figure 8. Respiration MG tomatoes cultivated under white LED light (WL) without- (**A**,**C**) or with far red lighting during cultivation (**B**,**D**) indicated as oxygen consumption (**A**,**B**) or CO_2 production (**C**,**D**) measured during cold storage at 2 or 12 °C. Orange, green, red and purple lines and symbols indicate 1, 5 and 21 kPa O_2 (low oxygen control), and 21 kPa O_2 at 12 °C (temperature control), respectively. The average O_2 or CO_2 production rates with indicated standard error are shown for two replicates from each respective container ($n = 2$) during cold storage at 2 °C or 12 °C. LSD values ($P < 0.05$) are indicated per panel.

H_2O_2 levels were stable during cold storage and steadily increased in all treatments during subsequent shelf life ($P < 0.0001$, Figure S3). Varying oxygen levels during cold storage showed similar patterns of H_2O_2 production during subsequent shelf life.

5.2.4. Discussion

5.2.4.1. Low oxygen storage alleviated CI in tomato which might be related to lower oxygen uptake and improved lycopene synthesis

When low temperature was combined with reduced oxygen concentrations, lower decay and lower weight loss was observed during shelf life for both MG and R tomatoes (Figure S1 and S2). Our results showed that O_2 consumption decreased with lower oxygen levels while CO_2 production rates were similar (Figure 8). Low oxygen storage is reported to suppress respiration and ethylene production (Klieber et al., 1996; Beaudry, 2000). Low oxygen uptake might reduce O_2 availability for ROS production, such as singlet oxygen ($^1O_2$) and superoxide anions ($O_2^{\cdot-}$) (Fahmy and Nakano, 2014). $O_2^{\cdot-}$ is dismutated into H_2O_2 by the action of SOD (Fahmy and Nakano, 2014; Imahori et al., 2016; Malacrida et al., 2016). Lower levels of $O_2^{\cdot-}$ are expected to yield lower levels of H_2O_2. However, we did not observe a lower level of H_2O_2 in the low oxygen stored fruit (Figure S3), perhaps indicating that low oxygen did not suppress oxidative stress initiated by the presence of $O_2^{\cdot-}$. As tomato stored under low oxygen showed further red colouration close to or even higher than the non-chilled control (Figure 4B) and faster red colouration (Figure 7) after transfer to 20 °C, we hypothesise that lycopene acted directly to quench $^1O_2$. Carotenoids are able to quench $^1O_2$ due to its high number of conjugated double bonds, whereas lycopene and its precursors, are the most effective $^1O_2$ quencher (Di Mascio et al., 1989; Min and Boff, 2002; Lado et al., 2016; Farneti et al., 2012). Quenching of $^1O_2$ by lycopene or its precursors might have resulted in delayed lycopene synthesis or lycopene degradation (Schouten et al., 2014; Farneti et al., 2014). Therefore, uninterrupted colour synthesis might indicate that low oxygen prevents lycopene degradation as well as preserving the lycopene biosynthetic machinery during cold storage allowing new lycopene synthesis during shelf life (Gartska et al., 2007; Yang et al., 2009; Skupien et al., 2017).

The lowest oxygen concentration to delay or prevent CI symptoms was 1 kPa (Figure 7). A lower oxygen level (0.5 kPa) resulted in necrosis and fungal infection (data not shown), probably because of excessive fermentation. It was reported that MG 'Bermuda' tomatoes stored at 22 °C under 0.5 kPa O_2 developed identical symptoms after three d of storage (Klieber et al., 1996).

5.2.4.2. Effects of low oxygen is larger to prevent CI related symptoms when tomatoes are cultivated with FR

Tomatoes cultivated with FR during cultivation and kept at 1 kPa O_2 during cold storage were shown to completely alleviate CI symptoms (Figure 5B) in MG fruit. This confirmed our previous findings that FR addition during cultivation suppressed CI incidence (Affandi et al., 2020). It was observed that MG tomatoes cultivated with FR initiated colour development at higher firmness (Affandi et al., 2020). It means that tomato cultivated with FR, although they had the same firmness as those cultivated without FR at harvest, maintained higher firmness

during cold storage (Figure 6). Excessive firmness loss during cold storage and during shelf life is often regarded as one of the main symptoms of CI in tomato with firmness retention associated with lower decay and higher membrane integrity (Mirdehghan et al., 2007; Rodoni et al., 2010). Improved cold tolerance of FR cultivated tomatoes might also be attributed to thicker cuticle wax layers (Cozmuta et al., 2016) which in turn might lower the oxygen consumption rate (Figure 8). On contrary, no significant difference was on weight loss (Figure S3). This might be attributed to comparably high relative humidity during the shelf life (> 95% RH) which suppress weight loss induced-transpiration from the fruit (Bhowmik et al., 1992).

Our findings suggests that when low oxygen storage is applied to accompany long cold storage or transport, higher CI tolerance will result in shelf life extension when tomatoes are grown with FR in greenhouses or grown in the field characterised by a low red to far-red ratio.

5.2.5. Conclusions

This study assessed the application of low oxygen either alone or in combination with far-red cultivated tomatoes on CI development. Results obtained showed the efficacy of low oxygen in minimising CI in tomato. CI tolerance is improved when low oxygen storage of MG tomatoes is combined with FR lighting during cultivation, especially when stored at 2 °C. This is likely due to lower oxygen uptake that allowed for to uninterrupted lycopene production and less softening during shelf life for prior cold stored MG tomatoes kept at 1 kPa O_2 and 0 kPa CO_2.

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Supplementary figures

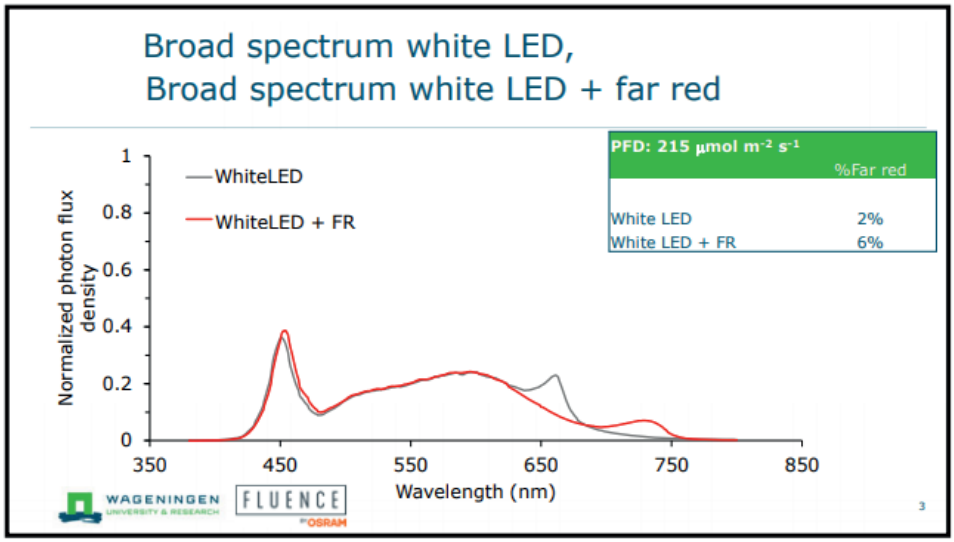

Figure S1. Spectra of the PhysioSpec Greenhouse lamp (A) with white LED light and far-red light and the spectra of the PhysioSpec Greenhouse lamp (B) with only white LED light. Total light intensity in µmol m^{-2} s^{-1}. Light intensity far-red light (A) = 12,6 µmol m–2 s–1. Light intensity far-red light (B) = µmol m^{-2} s^{-1}. PPF = photonsynthetic photon flux. YPF = yield photon flux. PPE = photosynthetic photon efficacy. R (625 – 700 nm) /FR (700 – 820 nm) = ratio between red and far red light.

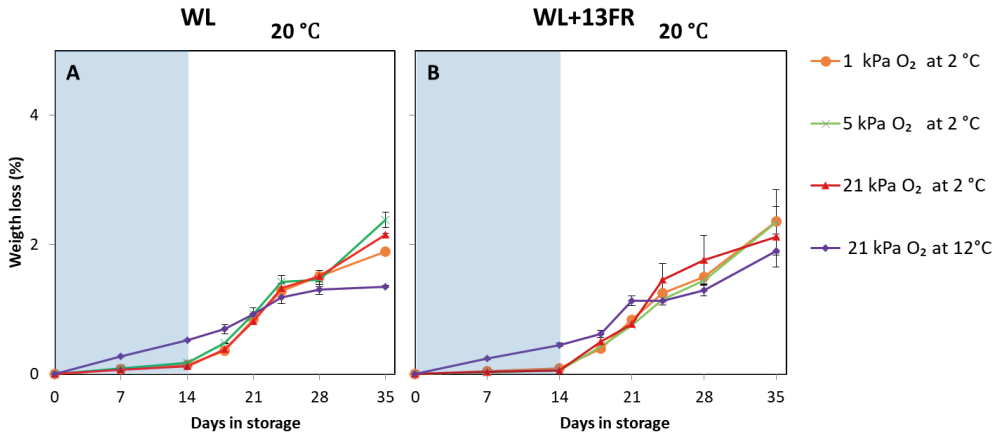

Figure S2. Weight loss percentage of MG (A) and R (B) tomatoes during cold storage at 2 °C (blue area) and subsequent shelf life at 20 °C (white area). Orange, green, red, and purple lines and symbols indicate 1 kPa, 5 kPa, 21 kPa O_2 (low oxygen control) applied during cold storage at 2 °C , and 21 kPa O_2 at 12 °C (temperature control), respectively. The average weight loss percentage with indicated standard error is shown for two replicates of four tomatoes (*n*=2)

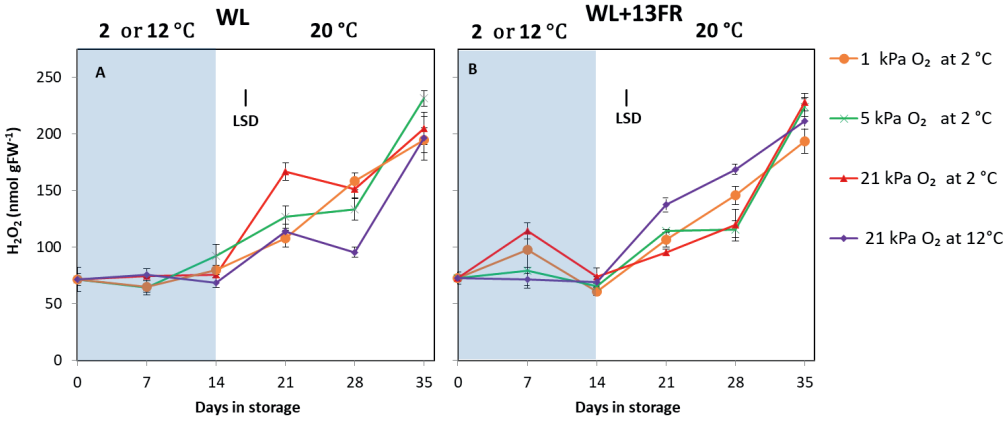

Figure S3. Hydrogen peroxide (H_2O_2) production of MG tomatoes cultivated under white LED light (WL) without far red lighting (A) or with additional far red lighting during cultivation (B) during cold storage (blue area) at 2 °C and subsequent shelf life at 20 °C (white area). Orange, green, red, and purple lines and symbols indicate 1 kPa, 5 kPa, 21 kPa O_2 (low oxygen control) applied during storage at 2 °C, and 21 kPa O_2 at 12 °C (temperature control), respectively. The average H_2O_2 production with indicated standard error is shown for two replicates of four tomatoes (*n*=2); (repeated measure over times). LSD values ($P < 0.05$) are indicated per panel.

CHAPTER

6

General discussion

Due to chilling injury (CI), tomato fruit do not benefit from low temperature storage to prolong shelf life, maintain fruit quality, and reduce postharvest losses. Tomato and several other fruit grown in tropical or sub-tropical origin are chilling sensitive as a result of their genetic make-up and growing conditions (light, temperature, water status, and relative humidity). Chilling sensitivity at harvest i.e. the risk of developing disorder due to low temperature may develop into CI problems in the postharvest stage. Therefore, a variety of postharvest techniques and treatments intended to prevent CI have been described (Parkin et al., 1989; Wang, 1993; Sevillano et al., 2009; Luengwilai et al., 2012; Aghdam and Bodbodak, 2014; Jannatizadeh et al., 2019). However, the mechanism that is responsible for sensitivity developing into CI has not been elucidated. Recently, the idea was proposed that preharvest conditions could also be of importance to reduce CI. To our knowledge, there is very little information on how cultivation circumstances affect chilling sensitivity. In addition, the interaction of postharvest treatments with chilling sensitivity, has not been systematically investigated.

The work presented in this thesis is intended to enrich current knowledge and understanding on the role of pre- and postharvest factors in chilling injury development in tomato. This work focused in particular on factors that have not yet been described:

- The role of preharvest light treatment (Far-red and blue LED) on chilling sensitivity (Chapter 2 and chapter 3)
- The effect of growing temperature on postharvest quality including the sensitivity and tolerance to cold storage (Chapter 4)
- The effect of low oxygen storage and its interaction with preharvest FR lighting in limiting CI (Chapter 5)

Results outlined in the chapters of this thesis demonstrate that CI symptoms in green and red tomatoes have some commonalities but are most of the time different. Observed CI symptoms in mature green (MG) tomatoes are uneven ripening, pitting, increased weight loss and faster softening. In red (R) tomatoes, the main CI symptoms are loss of red colouration, weight loss, and faster decay. Our findings agree with previous findings on CI in tomato (Lurie and Sabehat, 1997, Lurie and Crisosto, 2005; Farneti et al., 2012; Biswas et al., 2016; Albornoz et al., 2019). We investigated the effects of preharvest and postharvest factors on chilling injury development in both MG and R tomato. The effect of those treatments on a number of physiological processes associated with quality deterioration and CI defence mechanisms in tomato such as colour development, cellular integrity and defence against oxidative stress are discussed. The mechanisms behind some of the findings are currently not fully understood, and are interesting targets for future research.

6.1. The effect of preharvest conditions on tomato CI development might be explained by fruit physicochemical properties

Our LED light and temperature experiments revealed the importance of fruit physicochemical properties such as firmness and colour. Higher firmness at harvest may explain how moderate BL and additional FR induced cold tolerance on R tomatoes (Chapter 2 and Chapter 3). Reduced growth temperature improved chilling tolerance of dwarf MG tomatoes (Chapter 5). Even though a delay in red colour development indicated by a delay in crossing point (CP) was observed, an appreciable and uniform red colour was achieved after rewarming (Chapter 5). Improved cold tolerance of tomatoes grown at a reduced temperature was also associated with higher firmness at harvest and better firmness retention throughout cold storage and shelf life. The effects of each preharvest treatment and possible mechanisms underlying cold tolerance induced by those treatment will be discussed below.

6.1.1. Are the effects of preharvest light conditions on postharvest cold tolerance in R tomatoes mediated by fruit firmness?

Reduced softening and water loss may play an important role with regard to cold tolerance. Higher firmness at harvest acquired by FR and BL cultivated R tomatoes was maintained during postharvest storage under chilling temperature. Weight loss, colour, and decay index measurements indicated that tomatoes with higher firmness at harvest performed better during chilling and subsequent shelf life (Chapter 2, 3 and 5). Because CI aggravates the risk of fungal infections (Efiuvwevwere et al.,1988; Artes and Escriche, 1994; Mejía-Torres et al., 2009) and softening is contributed by reduced cell wall strength leads to infection (Wei et al., 2018), a treatment that retains firmness implies the cell walls are intact and less susceptible for infections (Sabban-Amin et al., 2011).

Firmness retention and reduced water loss of FR-treated fruit is presumably mediated by stimulation of wax biosynthesis in the cuticle, resulting in a tighter cuticle-wax adhesion and enhanced barrier properties against transpiration (Cozmuta et al., 2016). Softening in tomato is mainly contributed by catabolism of cell wall components and reduction in cellular turgor pressure (Saladie et al., 2007; Rugkong et al., 2011; Biswas et al., 2014). Increased firmness at harvest (Chapter 2), as well as a delayed softening during chilling and subsequent shelf life (Chapter 2 and Chapter 4) of FR cultivated tomatoes indicates that FR decreases the softening rate during tomato chilling, although previous reports mentioned the opposite (Alba et al., 2000). A low R:FR ratio or illumination of tomato seedlings with extra FR light induced the expression of the genes that play role in the metabolism of cell wall carbohydrates and in auxin responses (Cagnola et al., 2012). In tomato, auxin has been found to affect fruit firmness by regulating pectin structure and tissue architecture, although the molecular mechanism is unknown (Guillon et al., 2008). In addition, Wang et al. (2016) reported that FR and red light perceived by phytochrome A (phyA) and phytochrome B (phyB) regulate abscisic acid (ABA) and jasmonic acid (JA) signaling. FR also regulates CBF genes that further regulate the expression levels of cold responsive (COR) genes which convers chilling tolerance in plants by

stabilising the membranes during cold stress (Shi et al., 2017; Rihan et al., 2017). Most of these features which show FR effects on cold tolerance have not been studied in fruit. We therefore hypothesize that the effects of FR light during cultivation on chilling tolerance of red fruit postharvest may be due to changes in hormonal status and subsequent changes in cell wall properties.

Our result show that addition of BL during cultivation resulted in better cold tolerance. A moderate addition of 12% BL was the optimum level to induce cold tolerance. In addition, high intensity of preharvest BL (24%), showed sensitivity to cold storage, but to a lower extent than without added BL (0%). Interestingly, apart from differences in firmness and colour, none of the BL treatments resulted in higher ROS scavenging properties at harvest. Instead, differences in redox status indicators such as ascorbic acid (AsA), catalase (CAT), malondialdehyde (MDA) and dehydroascorbic acid (DHA) were affected by maturity but not by the preharvest BL conditions. In addition, the redox status indicators showed little changes throughout cold storage and shelf life independent of BL treatment. Postharvest BL treatments on detached fruit or vegetables were found to successfully enhance accumulation of the antioxidant pool (Li and Kubota, 2009; Avercheva et al., 2014; Shi et al., 2014; Xu et al., 2014; Naznin et al., 2016). Perhaps if the BL treatment would have been added during postharvest, our tomatoes would also show similar responses and convers more protection to chilling stress.

The reason for the lack of added BL during cultivation on antioxidant properties in our study is not clear and requires follow up research. A possible explanation could be attributed to lower overall light intensity in our experiment (175 μmol s^{-2}). Zushi et al. (2020), concluded that a higher intensity of BL (230 m^{-2} s^{-1}) is necessary to increase AsA accumulation *in vitro.* The spectrum is also an important factor. Ntagkas et al. (2019), reported that added BL light increased the antioxidant (AsA) accumulation in tomato fruit more than other light (including FR) did. We demonstrate that both BL and FR lighting induced a certain degree of cold tolerance (Chapter 2 and 3). Lower weight loss and continued colour development were observed for MG and R fruit cultivated with additional FR and only (partially) observed for R tomatoes cultivated with additional BL. This basically suggests that enhancement of antioxidants or the redox status at harvest is not the only factor determining chilling sensitivity. Instead, results from our study indicated that physiological maturity and physicochemical properties at harvest play a more important role in governing chilling tolerance compared to antioxidant properties.

6.1.2. De-synchronisation of colour and firmness is a typical CI symptom in MG tomatoes

It is widely accepted that green tomatoes are more chilling susceptible than red tomatoes (Autio and Bramlage, 1986; Lurie and Sabehat, 1997; Chomcalow et al., 2000; Biswas et al., 2012). However, this thesis demonstrated that MG tomatoes have less severe CI symptoms than R tomatoes (Chapter 3 and 5). This thesis also show that one common symptoms in MG tomatoes is de-synchronisation of colour development and firmness loss (Chapter 2, 4, 5). De-

synchronisation was indicated by rapid softening and continuous chlorophyll degradation and a lack of red colour development, leading to a delay in colouring. Red colour in tomato is mainly contributed by lycopene (Brandt et al., 2006).

FR and BL treatments did not result in higher firmness at harvest for MG tomatoes (Chapter 2 and 3). Nevertheless, cold tolerance of FR and BL tomatoes was characterised by higher firmness during cold storage and subsequent shelf life. Conflicting results were reported regarding the effect of chilling on the activities of cell wall degradation enzymes (Biswas, 2014). Chilling retarded activity of PG, β-galactosidase and pectate lyase (PL), but not PME (Jackman et al., 1992). It was also reported that enhanced softening during shelf life of tomatoes was not correlated with PG activity; instead, it was associated with PME activity (Marangoni et al., 1995). On contrary, Rugkong et al. (2011) did not find that cold storage retard PME activity in tomato. Results in this thesis outlined that softening of MG tomatoes already occurred during chilling (Chapter 2, 4 and 5) which might point to loss of turgor pressure playing a bigger role in softening during chilling than dissolution of cell wall components. Lower weight loss experienced by chilled MG tomatoes might be related to this. Turgor pressure in fruit is determined by the water status within the fruit and the degree of bound and free water inside the cell (Shackel et al., 1991; Jackman et al., 1992; Song et al., 2009). In tomato, increase in water mobility can be associated with cellular damage and increased membrane permeability (Albornoz et al., 2019). CI induced membrane breakdown resulted in higher water mobility leading to softening in persimmon (Clarck and Forbes, 1994), cherry fruit (Zhu et al., 2018), and bell pepper (Kong et al., 2018).

The chloroplast might be the first organelle suffering from CI that may lead to inhibition of colour development (Garstka et al., 2007; Skupień et al., 2017). Therefore, it is likely that uninterrupted lycopene synthesis depicted by chilling tolerant MG tomatoes from our FR, BL, growth temperature, and low oxygen experiments (Chapter 2, 3, 4 and 5) indicated a better chloroplast integrity enabling the transition from chloroplast to chromoplast (Yang et al., 2009; Skupień et al., 2017). Treatments that showed cold tolerance (Chapter 2, 3, 4 and 5) also maintained synchronisation between firmness loss and colour development throughout cold storage and subsequent shelf life. This thesis demonstrated that softening, weight loss, and colouring delay are interconnected and represent typical CI symptoms in MG tomatoes.

This thesis showed that some biochemical processes are not completely stopped during chilling. MG tomato either beefsteak type cultivars or dwarf cultivars, chilled at 4 °C for 15 d are still able to develop colour upon rewarming at 20 °C (Chapter 4, 5). This indicates that pigment synthesis is severely inhibited under low temperature but is reactivated upon rewarming. During cold storage, enzyme activities are low and enzymes involved in lycopene synthesis might be broken down that need to be resynthesized upon rewarming which causes delayed red colour formation (Chapter 5). The importance of lycopene and lycopene precursor synthesis is demonstrated by the fact that mature green tomato with delayed lycopene formation showed less CI symptoms (Chapter 2, 3 and 5). Precursors of lycopene, i.e. phytoene

and phytofluene, exhibit antioxidant capacity due their high number of conjugated double bonds (Martinez et al., 2014; Engelmann et al., 2012). It is therefore likely that delayed red colour synthesis also occur because of disruption of lycopene synthesis as lycopene or lycopene precursor are involved in scavenging excess ROS.

6.2. Critical reflection on oxidative stress a major cause of CI

Oxidative stress has long been considered as the key event preceding the onset of CI symptoms (Hariyadi and Parkin, 1991; Hodges et al., 2004; Valenzuela et al., 2019). Chilling stress provokes the imbalance between ROS production and ROS scavenging which leads to alterations in membrane structure and composition, membrane dysfunction, disruption of metabolic processes which finally results in the development of CI symptoms (Mittler, 2002; Demidchik, 2015).

Superoxide anion or oxygen radicals (O_2^-) and singlet oxygens ($^1O_2$) are ROS resulting from chilling stress which trigger oxidative stress (Watkins et al., 2017). The discussion on the relation between oxidative stress and chilling injury so far focused on oxidative stress triggered by the superoxide anion (Hodges et al., 2004; Sevilano et al., 2009; Singh et al., 2013). Cold stress causes an oxidative burst and activates NADPH oxidases in order to produce O_2^- which is then dismutated to H_2O_2 by super oxidase dismutase (SOD) (Das, and Roychoudhury, 2014). The role of H_2O_2 is important because it activates several antioxidants enzymes such as CAT, glutathione peroxidase (GPX) and ascorbate peroxidase (APX), or non-enzymatic antioxidants such as AsA (Gough and Cotter, 2011). Regeneration of AsA involves many enzymes such as monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Steffens et al., 2008; Imahori et al., 2016). Up-regulation of enzymatic or non-enzymatic antioxidants are perceived as a common denominator for chilling tolerance (Fig. 6.1). An association between up-regulation, accumulation of antioxidants and CI incidence has been established (Sevillano et al., 2009; Singh et al., 2013; Aghdam and Bodbodak, 2014; Valenzuela et al., 2017).

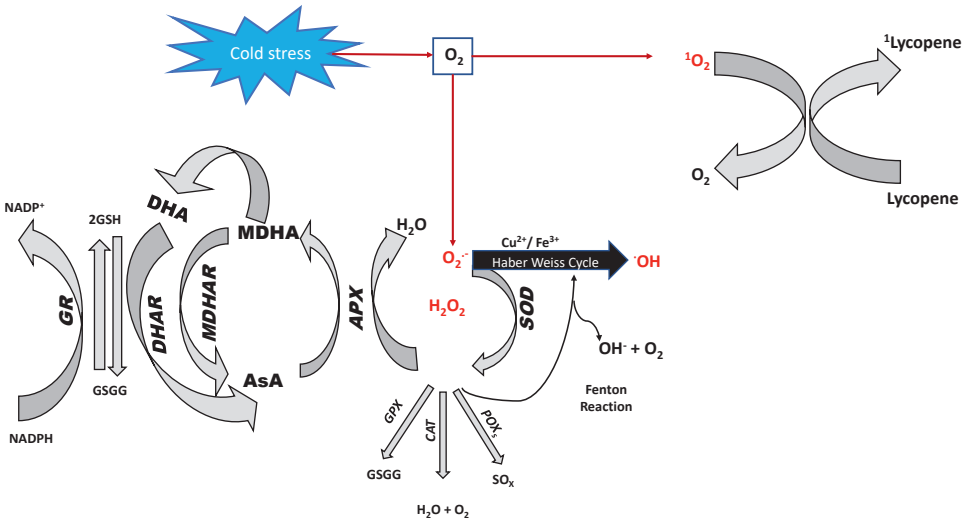

Figure 6.1. The generation of ROS and ROS scavenging compounds either enzymatic or non- enzymatic in tomato fruit. Chilling is thought to induce $O_2^{\cdot-}$ or $^1O_2$. The formation of $O_2^{\cdot-}$ may trigger production of H_2O_2 facilitated by SOD. Production of H_2O_2 trigger activation of antioxidant defence mechanisms such as AsA, APX, CAT, GR, MDHAR, DHAR and GSSG. The final ROS formed from this cascade of reaction is the most reactive ROS, $^{\cdot}OH$. In our research none of the events preceded by generation of $O_2^{\cdot-}$ seemed to be associated with chilling injury and it is hypothesized that an alternative pathway involving $^1O_2$ may be at play (adapted from Singh and Tuteja, 2010).

We could not find a clear relation between the level of non-enzymatic and enzymatic antioxidants such as vitamin C, CAT (Chapter 3) and SOD (data not shown), lipid peroxidation products such as MDA (Chapter 3) and ROS (Chapter 5) on the one hand with severity of CI on the other hand. Increased MDA levels or ion leakage, which is normally considered as a sign of the loss of membrane integrity due to lipid peroxidation, took place after 17 d storage at 4 °C (Sanchez-Bell et al., 2012; Biswas et al., 2012). In our research, however, 20 d cold stored (4 °C) tomato fruit - which showed complete inhibition of colouration - did not show an increased level of MDA. Even after an additional 15 d at post storage shelf life conditions, no clear increases in MDA were observed (Chapter 3). BL did not result in AsA accumulation at harvest and also no increase was found in response to postharvest chilling (Chapter 3). CAT activity decreased during chilling which indicates that CAT did not response to the chilling stress. Higher levels of H_2O_2 were not observed (Chapter 3). Absence of these redox status indicators during chilling may indicate oxidative stress initiated by singlet oxygen plays a more important role in CI development observed in our research. Unlike superoxide anion, the involvement of oxidative stress induced by singlet oxygen in chilling injury development has not been studied extensively.

6.3. Does low oxygen storage suppress CI in tomato due to lowering the presence of singlet oxygen?

The role of $^1O_2$ as a trigger for oxidative stress has not been elaborated in literature as it is unlikely to be produced during dark storage of fruit (Wise and Naylor, 1987; Lado et al., 2016). Singlet oxygen presence is associated with inefficient light energy dissipation in the presence of chlorophyll (Demidchik, 2015). Nevertheless, several reports mention that singlet oxygen is also detected in darkness in non-photosynthetic tissue under multiple stresses, such as drought and therefore might also be present due to temperature stress (Mor et al., 2014; Dmitrieva et al., 2020). $O_2^{\cdot-}$ induced oxidative stress causes damage to the cell through a cascade of reactions involving intermediate compounds such as H_2O_2, as well as scavenging antioxidants such as SOD, CAT, APX, AsA, GR and GSSH (Hossain et al., 2015). The final ROS resulted from this cascade is hydroxyl radical ($^{\cdot}OH$) via Haber-Weiss cycle under the presence of Cu or Fe ions (Fig. 6.1). Unlike the superoxide anion, singlet oxygen induces oxidative stress more directly, similar with the action of $^{\cdot}OH$ (Fig. 6.2). The unpaired electron of singlet oxygen makes it highly reactive and able to diffuse over several hundred nanometres causing direct damage to important biomolecules such as proteins, unsaturated fatty acids, and DNA, causing cell death that eventually leads to CI symptoms (Fischer et al., 2013; Pospišil and Prasad, 2014; Lado et al., 2016). Recently, the work of Lado et al. (2016) and Rey et al. (2020) showed that singlet oxygen and the fruits capacity to scavenge this radical species plays an important role in chilling susceptibility in carotenoid-rich fruit. However, the mechanisms of how chilling stress induced singlet oxygen production and its consequences on CI development is not elaborated by the authors.

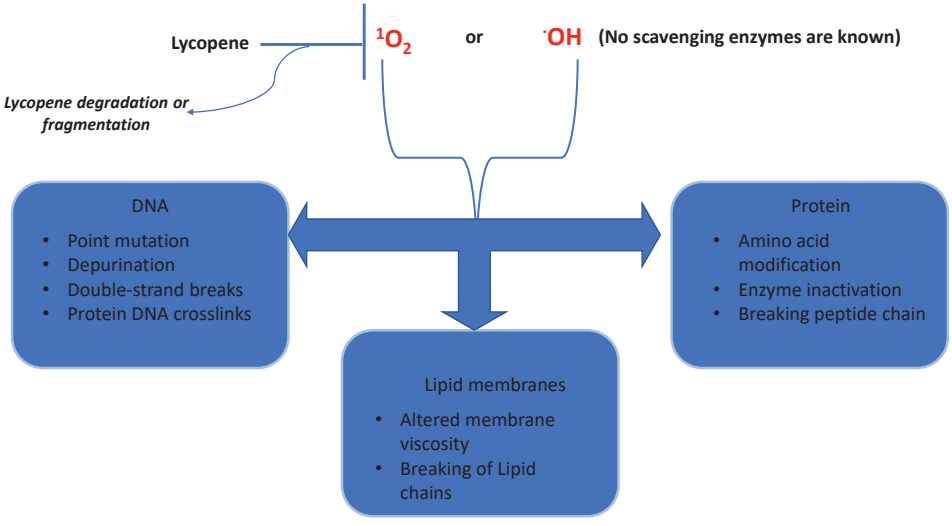

Figure 6.2. Various singlet oxygen and hydroxyl radical targets. Both ROS are able to create damage to DNA, lipid membranes and protein (adapted from Das and Roychoudhury, 2014).

The source of $^1O_2$ production in darkness could be hydroperoxides of fatty acids produced by lipoxygenase (LOX) from linoleic acid (Miyamoto et al., 2003; Miyamoto et al., 2013; Prado et al., 2020) (Fig. 6.3). Membrane rigidification as an initial response due to prolonged chilling may increase activity of phospholipase D (PLD) and LOX (Mao et al., 2007; Canone et al., 2011). Increase of LOX activity was reported to initiate CI in cucumber, and maize seedlings (Pinhero et al., 1998; Mao et al., 2007). Oxidation of linoleic acid in tomato by LOX resulting in the production of hydroperoxides may eventually lead to the production of singlet oxygen according to Russel's mechanism in which two peroxyl radicals combine via an intermediate tetraoxide, generating an alcohol, a ketone, and singlet oxygen (Pospisil et al., 2011; Dmitrieva et al., 2021).

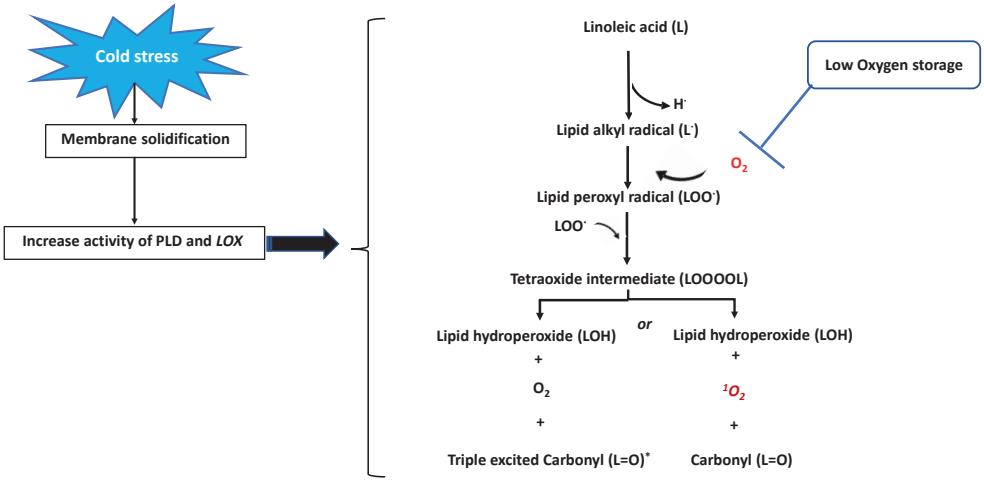

Figure 6.3. Possible explanation of singlet oxygen ($^1O_2$) generation by cold stress and how low oxygen storage may limit the generation of $^1O_2$ (adapted from Canone et al., 2011; Miyamoto et al., 2013).

Low oxygen storage decreased CI incidence in both MG and R tomatoes of round or cherry types (Chapter 5). It was shown that an atmosphere containing 2.5 kPa O_2 reduced chilling associated weight loss and decay in MG-, and prevented lycopene degradation in R tomatoes. Furthermore, we showed that even a lower oxygen level (1 kPa O_2) was more effective in alleviating visual CI symptoms. In terms of preventing lycopene degradation in red tomato, the effect of low oxygen storage is stronger than the applied preharvest light treatments such as FR (Chapter 2) and BL treatment (Chapter 3). In those light treatments, lycopene degradation upon chilling was still present, despite observable differences in other CI indicators. It was reported that heat shock treatment, which lead to the enhancement of ROS scavenging systems, applied to pink tomato, could not prevent lycopene degradation due to cold storage (Farneti et al., 2012). Lado et al. (2016) pointed out that increase in lycopene

content and not the activity of other antioxidant components contributed to better chilling tolerance of grapefruit.

Lycopene is a strong quencher of singlet oxygen, and the quenching takes place either by physical or chemical quenching (Ruth and Truscott, 2018). Physical quenching of $^1O_2$ by lycopene is based on energy transfer reactions. High energy level $^1O_2$ transfers its energy to the lycopene molecule to reach an excited state. Consequently, lycopene returns to its ground state by dissipating thermal energy to the surrounding or by isomerization. On the other hand, chemical quenching of $^1O_2$ or peroxyl radical by lycopene is responsible for cleaving long chain carbon skeleton of lycopene at one or both fragments resulting in lycopene derivatives such as apo-carotenals or carotenoid endoperoxides (Min and Boff, 2011; Wang, 2015; Heymann et al., 2015). Although chemical quenching of $^1O_2$ is less likely to occur than physical quenching (Heymann et al., 2015), it might still take place in our experiment. Subsequently, quenching of $^1O_2$ by lycopene potentially delays lycopene accumulation or leads to lycopene degradation to prevent further oxidative stress (Figure 6.3). This might explain why higher loss in red colouration of R tomatoes correlates with a lower degree of CI (Chapter 3). In addition, chilled storage under low oxygen led to less lycopene degradation in R tomatoes and sustained lycopene formation in MG tomatoes. This is presumably because of the suppression of chilling-induced singlet oxygen formation under low oxygen conditions (Chapter 5).

6.4. The ability to restore normal ripening after prior cold storage is an important feature of any treatment intended to alleviate CI

In MG tomatoes, apart from pitting, weight loss, and excessive firmness loss, the lack or delay in colouration during subsequent shelf life is one of the major chilling symptoms. This thesis highlighted that several preharvest treatments improved the chilling tolerance as reflected in the restoration of coloration (Chapter 2, 3, and 5). However, the outcome from the temperature experiment in which tomatoes were grown at other than the standard growing temperature demonstrated that chilling tolerance in terms of lower weight loss, less pitting and firmness retention can also be accompanied by delayed red colour formation (Chapter 4).

Since inability to ripen properly (full colour formation, synchronisation between colour development and firmness loss, flavour and aroma development, and biosynthesis of primary or secondary metabolites) is one of the important symptoms of CI, careful evaluation must be carried out to determine if any particular treatment sufficiently induces chilling tolerance. Slowing down ripening is the ultimate goal of many postharvest techniques. Delayed colour formation of MG tomato is often associated with longer storage and shelf life. In papers on CI in tomato, a delay of colouring was sometimes reported as a positive effect (Cantwell et al., 2009; Fagundes et al., 2015; Park et al., 2018). However, the inability to develop full red colour is one of main CI symptoms in tomato. It might be possible that the tomatoes experience delay in colour development will never fully become red. Therefore, delay in colouring in tomato must be interpreted more carefully.

The ability to restore normal ripening is an important feature of any postharvest treatment intended to reduce CI. Normal ripening of MG tomato is usually characterised by high synchronisation between colour development and firmness loss (Schouten et al., 2007; Saladié et al., 2014). Hence, any deviation from the normal ripening pathway in chilled MG tomatoes indicates CI. Consequently, chilling tolerance may be indicated by the closest possible colour development and firmness pattern to the normal ripening pattern. MG tomatoes suffering from CI usually show inability to develop sufficient colour accompanied by excessive softening. This results in poor ripeness at the end of shelf life (Albornoz et al., 2019). Therefore, treatments that restore colour formation for fairly firm tomatoes might indicate that tomatoes have potential to ripen (Chapter 2 and 3). From a practical point of view, treatments that alleviate CI and resulted in colour development retardation accompanied by firmness retention have the potential to withstands long transport to extended distribution ranges and attain full colour and desirable firmness at the end of the chain (Chapter 5). For shorter chains, chilling tolerance accompanied by faster colouration and firmness breakdown (Chapter 2, 3, and 4) is desired to allow for immediate marketing to reduce storage and inventory cost.

6.5. The role of low oxygen on lycopene metabolism at chilling conditions

The finding that low oxygen storage prevents lycopene degradation in red tomato during cold storage needs to be elucidated further. Several authors mentioned lycopene degradation as one symptom of CI in red tomato whereas in green tomato failure to synthesize red colour is mainly considered as the main CI symptom (Mejía-Torres et al., 2009; Farneti et al., 2012; Schouten et al., 2014). However, the reason and mechanism how postharvest cold stress causes lycopene degradation in red tomato is not clear. Lycopene breakdown is thought to be caused by scavenging of singlet oxygen or peroxyl radicals by lycopene. Low oxygen may affect production of these ROS (Figure 6.3). Therefore, more detailed studies which involve measurement of $O_2^{\cdot-}$ and $^1O_2$ as a function of low oxygen levels needs to be carried out to examine the role of low oxygen in inhibiting initiation of oxidative stress. Another option to measure $^1O_2$ would be to measure singlet oxygen absorbance capacity (SOAC) which assesses the total quenching activity of singlet oxygen by fruit or foods product (Ouchi et al., 2010; Iwasaki et al., 2015; Lado et al., 2016). Higher SOAC value might therefore point to higher singlet oxygen quenching power of lycopene. Comparing SOAC values with the activity of other antioxidants for tomatoes stored under low oxygen would clarify which ROS ($^1O_2$ and/or $O_2^{\cdot-}$) is involved in CI and which defence pathways operate under low oxygen conditions.

The benefit of applying controlled atmosphere (CA) storage for tomatoes relies on its ability to prolong the shelf life under favourable temperature conditions and to alleviate chilling symptoms under chilling conditions. In this way CA can either be applied to replace the need for low temperature storage or to alleviate chilling symptoms when fruit need to be stored together with chilling tolerant commodities at low temperatures. CA or modified atmosphere (MAP) applications in tomato are usually carried out at temperatures above CI threshold (Klieber et al., 1996; Sozi et al., 1999; D'Aquino et al., 2016). So far, a number of studies reported

that altering the storage atmosphere delayed or partially reduced CI symptoms in tomatoes (Forney and Lipton, 1990; Suparlan and Itoh, 2003; Cantwell et al., 2009; Cantwell and Saltveit, 2012; Fagundes et al., 2015; Deltsidis et al., 2018; Park et al., 2018). Unlike the mentioned studies, which applied oxygen level above 2.5 kPa, this thesis reported ultra-low oxygen (ULO) of 1 kPa can also effectively be used to reduce the adverse effect of chilling in tomatoes. Considering the effect of CA on ripening, this thesis revealed that low oxygen application during chilling may even accelerate colour development during shelf life of previously chilled MG tomatoes. This thesis hypothesised that reduction of CI in low oxygen storage is mainly due to suppression of singlet oxygen associated oxidative stress. Nevertheless, the known effect of CA on fruit physiology in general such as maintenance of higher level of antioxidant enzymes (Singh and Singh, 2013; Mditshwa et al., 2017) increased level of heat shock protein and ethanol (Sabban-Amin et al., 2011) and reduction of ethylene sensitivity (Orihuel-Iranzo et al., 2010) may synergistically induce chilling tolerance (Chapter 5).

6.6. Conclusions and future outlook

The work in this thesis gained insight into how preharvest factors such as light and temperature affect chilling sensitivity. We also investigated how low oxygen storage reduces CI symptoms. The main advancement provided by this thesis is that CI is also determined by the fruit's physicochemical properties (i.e., ability to retain firmness and restore colour and firmness synchronisation) which are greatly influenced by light and temperature during cultivation. The importance of lycopene and lycopene synthesis in CI mitigation is also highlighted. This thesis also indicated that one important feature of CA storage is avoidance of lycopene degradation, a distinct CI symptom in R tomato which cannot be alleviated by preharvest treatments. However, further studies are necessary to explore the physiological mechanisms at play when discussing light and temperature effects on chilling sensitivity and CI occurrence. This thesis also implied the existence of different pathways that mitigate CI in tomatoes. Identification of genes and induced pathways by preharvest and postharvest treatments, will advance our understanding on the underlying mechanisms that determine chilling sensitivity. Some other recommendation based on finding of this thesis are described below.

1. Tomato fruit cultivated in the greenhouse or a region characterized by low R:FR ratios will likely have a higher firmness at harvest and can be used for long distance transport or longer storage periods. The FR effect persists despite differences in light background (Chapter 2, Chapter 5). Since FR cultivated tomatoes can withstand cold storage (Chapter 2) or even can be cold stored longer under low oxygen storage (Chapter 5), it is a possibility to extent the marketing area. The applicability of this approach in tomato cultivation can be achieved especially in tomato grown in intercropping systems that are widely practiced in e.g. Indonesia. An intercropping system might be characterized by low R:FR ratio. For instance, it is now widely practiced to grow tomato plants under a chili pepper (*Capsicum annum*) crop, and it is reported that it gives higher yield (Rosliany and Setiawati, 2019).

However, to our knowledge, there are no reports on the performance of tomato harvested from this intercropping system during the postharvest phase, especially in the cold chain.

2. This thesis reveals that 1 kPa O_2 cold storage induced chilling tolerance in tomato. In general, an oxygen concentration below 2.5 kPa is not recommended (Cantwell and Suslow, 2002). Too low oxygen will shift respiration to fermentation which is a less efficient energy production route and may lead to production of unfavourable compounds such as ethanol and acetaldehyde. Therefore, research on CA or MAP application to mitigate CI in tomato usually evolve around safe oxygen level that avoid fermentation. However, chilling tolerance induced by CA storage might be due to the accumulation of low levels of ethanol and acetaldehyde, which might act as antioxidants. Chanjirakul et al. (2007) reported that fruit storage under ethanol vapour increased the radical scavenging capacity in strawberries and blackberries. Recently, ethanol treatment, either in the form of vapour or applied as a coating material was reported to alleviate CI symptoms such as internal browning in plums (Steffens et al., 2021) and astringency in persimmon (Fathi-Najafabdi et al., 2021). Therefore, we suggest further study into the effect of different ULO regimes on fermentative metabolism and redox status of the fruit. Comparing characteristic responses of tomato under hypoxia in combination with cold storage will provide insight on how fermentation is induced by ULO and which level of induced fermentation effectively suppresses CI.
3. Further study on lycopene metabolism under the combination of low temperature and low oxygen needs to be carried out. Measurement of lycopene breakdown products and lycopene precursors as well as enzyme activities in the carotenoid pathway will clarify whether lycopene degradation occurs or that the perceived lycopene degradation is merely an imbalance between lycopene synthesis and isomerisation to e.g., β-Carotene. Studying the activity of enzymes involved in lycopene degradation will also shed more light on the mechanism of low O_2 storage in preventing lycopene degradation.
4. The impact of CI on volatile profiles of tomato is widely mentioned in literature (Deltsidis et al., 2013; Farneti et al., 2015; Wang et al., 2015). However, this thesis has not addressed this issue. The dynamics in tomato volatile production during postharvest cold storage can be used to characterise tomato chilling injury. Volatile biosynthesis is affected by low temperature storage, and lower volatile emissions after cold storage have been reported (Bai et al., 2011; Farneti et al., 2015). During cold storage, tomato volatiles from lipid peroxidation pathways decrease whereas volatiles derived from carotenoid biosynthetic pathway tend to increase (Farneti et al., 2014). For instance, 6-methyl-5-hepten-2-one increased and it is likely related to decreased lycopene synthesis (Deltsidis et al., 2013). Volatile biosynthesis is mostly regulated at the transcript level (Verdonk et al., 2005), and many volatile biosynthetic genes have been characterized (Speirs et al., 1998; Chen et al., 2004; Simkin et al., 2004; Klee and Tieman, 2013; Shen et al., 2014; Goulet et al., 2015). These volatiles and genes might be used as CI markers. These markers can then be used to screen tomato genotypes for their suitability to benefit from CA or MAP storage.

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Summary

One of the major challenges faced by fresh produce from tropical origin is chilling injury (CI). Because of CI, the benefit of low temperature storage to maintain freshness and quality along the chain cannot be maximized. Alleviating CI will not only assure good performance of fruit and vegetable in the chain but also reduce postharvest loss and preserve nutritional sources for individuals, especially in developing countries. Therefore, efforts and studies are devoted to tackle CI in numerous fresh produce. Tackling CI problems needs synergistic approach that encompasses preharvest and postharvest factors. This thesis emphasis preharvest factors determining chilling sensitivity and postharvest factors that induce or alleviate CI.

Chapter 1 describes the state of the art with respect to the possible role of preharvest factors such as lighting and temperature in chilling sensitivity in tomato. The aim of this thesis, to explore multiple pathways involved in CI and the influence of preharvest and postharvest factors is discussed.

Chapter 2 describes the role of preharvest far-red (FR) LED light on postharvest cold tolerance in 'Moneymaker' tomato cold stored for maximally fifteen days followed by shelf life conditions for twenty days. Preharvest FR light induced cold tolerance in both MG and R tomatoes. In MG tomatoes, additional far-red light resulted in reduced weight loss, less pitting and faster red colour development during shelf life (when prior long cold stored), and less softening (when prior short or non-cold stored). We hypothesised that FR lighting during cultivation protects the membrane integrity of MG tomatoes which allows uninterrupted lycopene synthesis. In R tomatoes, preharvest FR lead to firmer fruit at harvest, and this contributed to reduced weight loss and less decay during shelf life. The study showed that induced cold tolerance by FR light applied during cultivation, might be related to FR induced cuticle wax biosynthesis and the action of lycopene as an antioxidant during cold storage. Finally, we envisage that FR lighting during tomato cultivation might have an important role in facilitating long distance transport at low temperatures.

Chapter 3 describes how preharvest blue LED lighting (BL) induces postharvest cold tolerance in 'Foundation' tomatoes. In this study chilling injury indices and important quality properties such as colour, firmness, hydrogen peroxide, malondialdehyde, ascorbic acid and catalase activity were characterised during cold storage at 4 °C for different durations followed by shelf life of twenty days. Acquired cold tolerance of R fruits harvested from the 12B lighting conditions was related to its ability to loose red colour, presumably in favour of the scavenging

of ROS. MG-tomatoes showed no CI symptoms, regardless of the preharvest lighting. No effects of light treatments were found on several antioxidant capacity indicators of both tomatoes. The red colour, as measured by remittance VIS spectroscopy, is closely related to the lycopene concentration. The idea that lycopene in 12B tomatoes is a more efficient antioxidant compared to that of the other BL treatments is put forward. It was also revealed that improved cold tolerance for R fruit was not due to differences in redox statues indicators (CAT activity, total ascorbic acid, H_2O_2 and MDA levels) but due to a lower red colour at harvest and faster discolouration during cold storage. Here we again showed that through an extensive cold storage experiment, the chilling injury syndrome can be assessed by quality properties behaviour analysis during the (after cold storage) shelf life employing non-destructive techniques.

Chapter 4 describes The effect of varying growth temperature (16, 22, 26 °C) on evolution of quality properties and CI development in dwarf cultivars (Ponchi Re and Tarzan) tomatoes grown in climate chambers. It is revealed that the lowest growth temperature (16 °C) performed better during shelf life and the effect of low growth temperature has a genetic component. We showed that low growth temperature delays the onset of red colour development during shelf life and the delay was more pronounced when fruit were first stored at low temperature. Cultivation at the lowest growth temperature resulted in fruit firmness retention during cold storage and shelf life and this was also corroborated with lower weight loss. Growth temperature affects CI development differently in the 2 genotypes. 'Ponchi Re' tomatoes showed less CI symptoms during after storage shelf life with higher growth temperatures, whereas the opposite was observed for 'Tarzan' tomatoes. We hypothesised that the delay in the start of the red colouration for 'Ponchi Re' tomatoes grown at 16 °C exposed the tomatoes (at chilling temperatures) to ROS without proper scavenging capacity provided by lycopene. For 'Tarzan' tomatoes higher CI tolerance of tomatoes cultivated at 16 °C might be induced by higher firmness at harvest and lower weight loss during storage. Taken together our study indicates that cold tolerance depends on growth temperature and cultivar in dwarf tomato fruit.

Chapter 5 describes the role of low oxygen storage in limiting CI development in tomatoes. The effect of low oxygen on cold tolerance were consistent in different tomato types. In **Chapter 5.1**. fruit from Cherry type cultivars were cold stored at 2 °C at varying oxygen levels. Applying low oxygen (down to 0.5 kPa) during cold storage was beneficial for cold tolerance of MG tomatoes. 5 kPa O_2 showed the best results for both MG and R tomatoes in terms of delayed softening, less decay and full colouration (MG) during the after storage shelf life. In **Chapter 5.2** the combination of FR and low oxygen-induced cold tolerance was tested. Depending on the experiment we investigated the role of low oxygen (0.5, 2.5 and 5 kPa O_2) in

limiting CI in round tomato cultivar for MG and R stage. Next we applied low oxygen (1 and 5 kPa O_2) storage on tomato cultivated under additional FR. Low oxygen induced cold tolerance in MG and R tomatoes. We showed that decay, firmness loss and weight loss during were reduced under influence of low oxygen. Red colour degradation of R tomatoes (a CI symptom) was suppressed under low oxygen. The effect of low oxygen was greater when applied to MG tomatoes that had been cultivated under additional FR due to a lower respiration rate that might resulted in low singlet oxygen formation. No clear relation was found between H_2O_2 level and the extent of CI. Therefore, we propose the idea that oxidative stress initiated by singlet oxygen and not superoxide anion plays a more pronounced role in inducing CI in tomato. Furthermore, we hypothesised that the formation of singlet oxygen was suppressed by low oxygen.

In **Chapter 6** the new understanding of the effect of pre- and postharvest conditions on tomato fruit postharvest CI development described in chapter 2-5 is discussed. Attention is given to the possible role of the fruit's physical properties in establishment of cold tolerance. New insight on oxidative stress as an important CI inducer is also discussed. That is, the role of singlet oxygen as oxidative stress initiator and the ability of lycopene to scavenge singlet oxygen is thought to be important for cold tolerance in lycopene rich fruit like tomatoes. Furthermore, the limitation of the studies presented in this thesis as well as consequences of the main findings and further research recommendations are discussed.

Ringkasan

Salah satu tantangan terbesar yang dihadapi oleh produk buah dan sayur tropis adalah *chilling injury* (CI) atau cedera karena pendinginan. Karena CI manfaat dari pendinginan untuk mempertahankan kesegaran dan kualitas produk sepanjang rantai pasok tidak dapat diraih secara maksimal. Upaya mengurangi CI tidak hanya akan menjaga mutu buah dan sayur tetapi juga mengurangi susut pascapanen dan mempertahankan sumber gizi yang terjangkau, terutama di negara berkembang. Karena itu, dalam upaya dan studi untuk mengatasi CI pada banyak produk, upaya sinergis yang mengintegrasikan faktor-faktor pra dan pascapanen sangat diperlukan. Disertasi ini memberikan penekanan pada faktor prapanen yang berpengaruh pada sensitivitas pendinginan dan faktor pasca panen yang mempercepat terjadinya CI.

Bab 1 memaparkan peranan faktor prapanen seperti pencahayaan dan suhu terhadap sensitivitas pendinginan buah tomat ditilik dari perkembangan riset terkini di bidang fisiologi tanaman. Bab ini juga mendiskusikan tujuan tesis ini untuk mengeksplorasi jalur-jalur reaksi yang terlibat dalam CI.

Bab 2 menjelaskan tentang peranan pencahayaan sinar *far-red* (FR) saat prapanen dalam menginduksi toleransi terhadap suhu dingin untuk tomat kultivar Moneymaker yang disimpan selama maksimal 15 hari diikuti penyimpanan *shelf life* (pada suhu 20 °C) selama 20 hari. Kami menemukan bahwa pencahayaan prapanen dengan sinar FR membuat tomat hijau maupun merah lebih tahan suhu dingin. Pada tomat hijau ketahanan ini ditunjukkan dengan susut bobot dan perlubangan yang lebih rendah seiring dengan perubahan warna menuju merah yang lebih cepat (setelah tomat didinginkan pada durasi yang lama) dan ketegaran yang tetap terjaga (setelah pendinginan pada durasi singkat atau tanpa pendinginan). Hipotesis kami, sinar FR melindungi integritas membran pada tomat hijau yang memungkinkan sintesis likopen yang tidak terputus. Pada tomat merah, sinar FR prapanen menghasilkan tomat dengan ketegaran yang lebih tinggi saat panen yang berkontribusi positif bagi rendahnya susut bobot dan pembusukan selama *shelf life*. Studi ini menunjukkan bahwa peningkatan toleransi akibat sinar FR dapat disebabkan oleh perubahan kimiawi pada kutikula tomat dan aktivitas likopen sebagai antioksidan selama pendinginan. Akhirnya, kami memaparkan kemungkinan bahwa pencahayaan FR selama budidaya tomat mungkin memiliki peran penting dalam memfasilitasi transportasi jarak jauh pada suhu rendah.

Bab 3 menjelaskan bagaimana pencahayaan prapanen dengan lampu LED biru (BL) menginduksi toleransi dingin pascapanen pada tomat 'Foundation'. Dalam studi ini, indeks cedera dingin dan parameter mutu penting seperti warna, ketegaran, hidrogen peroksida, malondialdehid, vitamin C, dan aktivitas katalase dikarakterisasi selama penyimpanan dingin

pada suhu 4 °C untuk durasi yang berbeda diikuti oleh penyimpanan *shelf life* selama dua puluh hari. Buah tomat merah yang dipanen dari kondisi pencahayaan 12B lebih toleran terhadap suhu dingin yang terkait dengan kemampuannya untuk menetralisir *Reactive Oxygen Species* (ROS) yang ditunjukkan dengan kehilangan warna merah. Tomat hijau tidak menunjukkan gejala CI, terlepas dari pencahayaan prapanen. Tidak terdapat efek perlakuan pencahayaan terhadap beberapa indikator kapasitas antioksidan dari kedua tomat. Warna merah, yang diukur dengan spektroskopi VIS remitansi, terkait erat dengan konsentrasi likopen. Kami juga menawarkan hipotesis bahwa likopen dalam tomat 12B merupakan antioksidan yang lebih efisien daripada tomat pada perlakuan BL lainnya. Juga terungkap bahwa peningkatan toleransi dingin untuk tomat merah tidak disebabkan oleh perbedaan indikator redoks (aktivitas CAT, total asam askorbat, kadar H_2O_2 dan MDA) tetapi karena warna merah yang lebih rendah saat panen dan perubahan warna yang lebih cepat selama penyimpanan dingin. Di sini kami sekali lagi menunjukkan bahwa melalui eksperimen penyimpanan dingin yang ekstensif, gejala CI dapat dinilai dengan analisis karakteristik kualitas selama masa simpan (setelah penyimpanan dingin) menggunakan teknik non-destruktif.

Bab 4 menjelaskan pengaruh pelbagai suhu pertumbuhan (16, 22, 26 °C) terhadap mutu dan CI pada tomat kultivar kerdil (Ponchi Re dan Tarzan) yang ditanam di *climate chamber*. Terungkap bahwa tomat dari suhu terendah (16 °C) menunjukan performa lebih baik selama *shelf life* dan efek suhu pertumbuhan ini dipengaruhi oleh unsur genetis. Kami menunjukkan bahwa suhu pertumbuhan yang rendah menyebabkan perlambatan sintesis warna merah selama *shelf life* dan penundaan ini menjadi lebih signifikan ketika buah terlebih dahulu disimpan pada suhu rendah. Budidaya tomat kerdil pada suhu 16 °C menunda pelunakan dan susut bobot tomat. Dua genotip dalam eksperimen ini menunjukan respon berbeda terhadap suhu pertumbuhan. Tomat 'Ponchi Re' menunjukkan gejala CI yang lebih rendah apabila ditanam pada suhu pertumbuhan yang lebih tinggi berkebalikan dengan tomat 'Tarzan'. Kami berhipotesis bahwa tertundanya sintesis warna merah pada 'Ponchi Re' yang ditanam pada 16 °C membuat tomat terpapar ROS selama pendinginan tanpa aktivitas antioksidan yang cukup dari likopen. Untuk tomat 'Tarzan', toleransi suhu dingin yang lebih tinggi dari tomat yang dibudidayakan pada 16 °C mungkin disebabkan oleh kekerasan yang lebih tinggi saat panen dan susut bobot yang lebih rendah selama penyimpanan. Penelitian kami menunjukkan bahwa toleransi suhu dingin pada tomat kerdil dipengaruhi oleh suhu pertumbuhan dan faktor genetis.

Bab 5 menjelaskan peran penyimpanan pada ruangan beroksigen rendah dalam menghambat perkembangan CI pada tomat. Pengaruh oksigen terhadap toleransi suhu dingin ini konsisten pada jenis tomat yang berbeda. Dalam Sub-bab 5.1. tomat *cherry* didinginkan pada suhu 2 °C

pada beberapa tingkat oksigen yang berbeda. Penerapan oksigen rendah (hingga 0,5 kPa oksigen) selama penyimpanan dingin berpengaruh positif terhadap tomat hijau. Penyimpanan pada oksigen 5 kPa menunjukkan hasil terbaik untuk tomat hijau maupun merah ditinjau dari terjaganya ketegaran buah, pembusukan yang melambat dan warna merah yang maksimal pada akhir masa penyimpanan. Pad Sub-bab 5.2, kami menguji efek kombinasi antara FR dan oksigen rendah terhadap toleransi suhu dingin. Pada percobaan awal kami menginvestigasi peranan oksigen rendah (0,5, 2,5 dan 5 kPa O_2) dalam menghambat CI pada tomat berukuran besar baik tomat merah maupun hijau. Selanjutnya kami menerapkan penyimpanan oksigen rendah (1 dan 5 kPa O_2) pada tomat yang dibudidayakan dengan FR. Kami menunjukkan bahwa pembusukan, pelunakan dan susut bobot jauh berkurang selama penyimpanan jauh berkurang pada kondisi oksigen rendah. Degradasi warna merah yang merupakan gejala khas CI pada tomat merah dapat ditekan pada kondisi oksigen rendah. Pengaruh oksigen rendah akan lebih besar apabila diterapkan pada tomat hijau yang telah dibudidayakan dengan tambahan sinar FR. Hal ini dapat diakibatkan tingkat respirasi yang lebih rendah yang berpotensi menekan pembentukan oksigen singlet. Kami tidak dapat menemukan hubungan yang jelas antara tingkat H_2O_2 dan keparahan CI. Oleh karena itu, kami berhipotesis bahwa pada tomat stres oksidatif yang disebabkan oleh oksigen singlet memainkan peranan yang lebih penting dalam perkembangan CI daripada stres oksidatif akibat anion superoksida.

Bab 6 membahas pemahaman baru tentang pengaruh kondisi pra dan pascapanen terhadap perkembangan CI pada pascapanen buah tomat yang dijelaskan dalam Bab 2-5. Disertasi ini juga mengemukakan wacana tentang pentingnya peranan sifat fisik buah terhadap toleransi suhu dingin. Perspektif baru tentang stres oksidatif sebagai penginduksi CI juga mendapatkan perhatian. Kami menilai bahwa peranan oksigen singlet sebagai inisiator stres oksidatif dan kemampuan likopen untuk menetralisirnya lebih penting tehadap toleransi suhu dingin pada buah kaya likopen seperti tomat. Selanjutnya, kami juga membahas keterbatasan studi yang disajikan dalam tesis ini serta konskuensi dan kemungkinan penerapan dari beberapa temuan penting dari disertasi ini.

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About the Author

Fahrizal Yusuf Affandi was born in Yogyakarta on 26 October 1981. Fahrizal obtained a bachelor degree in Agroindustrial technology from Universitas Gadjah Mada, Indonesia in 2005. After which he worked as a site manager in national project on reducing postharvest losses on crop and horticulture product until 2008. On 2009, he was awarded a fellowship from Vlir-Uos Belgium to pursue a master study in Food Technology at KU Leueven and Ghent University, Belgium. He successfully finished his master degree with specialisation in food preservation and postharvest technology. His master thesis title was 'Comparing Dynamic Controlled Atmosphere (DCA) Strategies for long time storage of Apple'. After finishing his master study, Fahrizal works as a lecturer in agroindustry department vocational college Universitas Gadjah Mada, Indonesia. He taught some subject related to food preservation technology, such as unit operation, engineering properties of agriculture product and postharvest technology of horticultural product. On 2015 Fahrizal was awarded a fellowship from Indonesian Endowment Fund for Education (LPDP) to conduct a PhD study in Wageningen University, the Netherlands. His research interest is postharvest physiology of fruit with focus on chilling injury of tomato, controlled atmosphere and relation between preharvest and postharvest factors on chilling injury development. Fahrizal is a member of International Society for Horticultural Science (ISHS) and Association of Agroindustrial Technologist (APTA, Indonesian). During his doctoral study, Fahrizal was highly involved in the special branch of Nahdlatul Ulama for the Netherlands where he was appointed as general secretary from 2017-2021.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- The role of preharvest factors and low oxygen storage on development of chilling injury in tomato

Writing of project proposal (4.5 ECTS)

- Minimizing chilling sensitivity and chilling injury in tomato

Post-graduate courses (6.1 ECTS)

- Basic statistics; PE&RC (2016)
- Design of experiments; PE&RC/WIAS (2016)
- Reaction kinetics in food; VLAG (2018)
- Metabolomics; Institute of Biology Leiden University (2019)
- Introduction to data science with R and Rstudio; PE&RC (2019)

Laboratory training and working visits (0.3 ECTS)

- Postharvest technology course excursion; Nedcool, Prominent, Ripening Center (2018)

Deficiency, refresh, brush-up courses (6 ECTS)

- Product quality measurements & analysis; HPP-WUR (2016)

Competence strengthening / skills courses (2.5 ECTS)

- Efficient writing strategies; WGS (2019)
- Ethics in plant and environmental science; WGS (2019)
- Scientific integrity; WGS (2020)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Day (2017, 2019)
- PE&RC Last year weekend (2019)

Discussion groups / local seminars or scientific meetings (5.7 ECTS)

- FLOP: Frontier Literature on Plant Physiology (2016-2019)
- Postharvest seminar with WUR Food and Biobased Research (FBR) (2018-2020)

International symposia, workshops and conferences (5.4 ECTS)

- Wageningen Indonesia scientific exposure (2017, 2021)
- Postharvest loss symposium-international horticultural congress (2018)
- XIIIth International Controlled and Modified Atmosphere Research Conference (2021)

Lecturing / supervision of practical's / tutorials (6 ECTS)

- Postharvest physiology practical (2016, 2017)

MSc thesis supervision

- Effect of preharvest blue LED on CI in tomato
- The role of water and temperature stress on tomato cold tolerance
- Finding optimum low oxygen concentration to minimise CI in tomato

List of Publications

Paper published in refereed journals

Affandi, F.Y., Verdonk, J.C., Ouzounis, T., Ji, Y., Woltering, E.J. and Schouten, R.E., 2020. Far-red light during cultivation induces postharvest cold tolerance in tomato fruit. Postharvest Biology and Technology, 159, 111019.

Affandi, F.Y.; Verschoor, J.A.; Paillart, M.J.M.; Verdonk, J.C.; Woltering, E.J.; Schouten, R.E., 2021.Low Oxygen Storage Improves Tomato Postharvest Cold Tolerance, Especially for Tomatoes Cultivated with Far-Red LED Light. Foods, 10, 1699. https://doi.org/10.3390/foods10081699.

Affandi, FY., Shiri, M., Woltering, EJ., Schouten, RE., 2021. Low Oxygen Storage Alleviates Chilling Injury in Cherry Tomatoes. Acta Horticulturae, *In press*

Paper submitted or in preparation

Affandi, FY., Prayoga, T., Ouzounis, T., Giday, H., Verdonk, JC., Woltering, EJ., Schouten, RE., Additional blue light during cultivation induces cold tolerance in tomato fruit, but only to an optimum. (*submitted*)

Affandi, FY., Pijnenburg, C., Verdonk, JC., Woltering, EJ., Schouten, RE., Growth temperature affects postharvest quality properties and chilling tolerance of green harvested dwarf tomatoes.

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