Effects of Postharvest Techniques on Nutritional Quality of Cherry Tomatoes

by

Yanjie Song

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Abstract

Cherry tomatoes play an important part in the human diet as they have a high content of nutritional component, including ascorbic acid, carotenoids and total phenolics. This study aimed to investigate how postharvest techniques, temperature, light irradiation and Modified atmosphere packaging (MAP) affected nutritional quality of cherry tomatoes, and to identify the strategies to preserve their quality.

Three temperatures (5, 15, 20°C) were examined to evaluate the effect of temperature on cherry tomatoes. A storage temperature of 15°C was found to inhibit weight loss and the decrease of lycopene, ß-carotene and lutein content when compared with 20°C, whereas a low temperature (5°C) caused chilling injuries, such as pour colour development, and inhibited the increase of lycopene and ß-carotene content compared with 15 and 20°C.

The effect of postharvest red/far-red (ratio 0.89) and blue light irradiation on fruit quality was also investigated. The results showed that red/far-red light inhibited weight loss, inducing colour changing from green to red, and increased the content of lycopene, β-carotene, and total phenolics compared to darkness. In contrast, blue light induced weight loss, and had little effect on colour change and the content of ascorbic acid, lycopene and total phenolics compared to darkness.

The influence of the combination of red/far-red light and modified atmosphere packaging on fruit quality was also examined. The results showed that the combined treatment effectively extended shelf-life of cherry tomatoes by delaying ripening as indicated by the delayed increase of respiration and colour change from green to red, reduced weight loss, and increased the content of lycopene and ß-carotene.

The role and mechanism of red/far-red light in the regulation of carotenoid biosynthesis were explored. Results showed that gene *Phytoene synthase* (*PSY*), *Zeta carotene* (*ZDS*) and *Chloroplast lycopene beta cyclase* (*LCY-b)* and were overexpressed in fruits treated with red/far-red light during storage compared to the darkness. Moreover, red/far-red light induced expression of *1-aminocyclopropane-1-carboxylate synthase 2* (*ACS2)* during the first 25 days of storage, *Ripening inhibitor* (*RIN*) during the first 28 days and *Elongated hypocotyl 5* (*HY5*) during the whole period of storage. This provided a hypothetical model of red/far-red light in the regulation of carotenoid biosynthesis.

List of Abbreviations

°C	Celsius
µL	Microlitre
µm	Micrometre
ACS	1-aminocyclopropane-1-carboxylate synthase
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ANOVA	Analyses of variance
B-L	Long-term blue light
B-M	Middle-term blue light
B-S	Short-term blue light
CA	Controlled atmosphere
cm	Centimetre
CRTISO	Carotenoid isomerase
DM	Dry mass
EIN3	Ethylene-insensitive 3
FW	Fresh weight
g	Gram
HSD	Honest significant difference
HY5	Elongated hypocotyl 5
LCYb	Lycopene β-cyclase
LCYe	Lycopene ε-cyclase
MAP	Modified atmosphere packaging
mg	Milligram
mL	Millilitre
mm	Millimetre
nm	Nanometre
ns	No significant difference
PDS	Phytoene desaturase
PHYA	Phytochrome-A
PHYB	Phytochrome-B
PIF3	Phytochrome-interacting factor 3
PSY	Phytoene synthase

R/FR	Red/far-red
R/FR-L	Long-term red/far-red light
R/FR-M	Middle-term red/far-red light
R/FR-S	Short-term red/far-red light
RIN	Ripening inhibitor
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SE	Standard error
TF	Transcription factor
USDA	United States Department of Agriculture
ZDS	ζ-carotene desaturase

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Declaration

I hereby declare that the work presented was carried out by the author except where specific reference is made to other sources. This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy, and it has not been submitted, in whole or in part, for any other degree, diploma or other qualification.

1. Introduction

1.1. Literature review

1.1.1. Botanical description and cultivation of tomato

The tomato, usually referred to as cultivated tomato (*Solanum lycopersicum* L.), belongs to the Solanaceae family, which consists of ~98 genera and ~2700 species, including potatoes, eggplant and bell peppers (González et al., 2011; Olmstead and Bohs, 2006). It is a member of *lycopersicon* section along with 12 wild relatives: *S. chilense*, *S. arcanum*, *S. chmielewskii*, *S. neorickii*, *S. corneliomulleri*, *S. pennellii*, S. *peruvianum*, *S. cheesmaniae*, *S. galapagense*, *S. habrochaites*, *S. huaylasense* and *S. pimpinellifolium* (Knapp and Peralta, 2016).

Tomatoes are usually divided into five major types: plum, classic round, beefsteak, cherry and cocktail, and vine or truss based on their size, taste, flavour, colour and nutritional compounds (Table 1.1) (González et al., 2011; Heuvelink, 2018).

Table 1.1 Tomato types, characteristics and uses (Heuvelink, 2018).

Type	Use
Plum tomatoes	Grilling and processed for pasta dishes and pizzas
Classic round tomatoes	Salads, or for baking, grilling, or frying, and as ingredient for soups or sauces
Beefsteak tomatoes	Stuffing and baking whole, and sliced for sandwiches and salads
Cherry and cocktail tomatoes	Eaten whole and raw or cooked. Halved in salad or skewered whole for grilling
Vine or truss tomatoes	This may be any type mentioned above, but marketed attached to the stem, which gives the distinct tomato aroma

Cherry tomato (*L. lycopersicum* var. *cerasiforme*) is one of the most popular types of tomatoes because of its smaller size, succulent taste and high nutritional quality, making them

excellent for fresh eating (Liu et al., 2018). The ancestor of modern cultivated varieties was most likely native to the Peru-Ecuador area, and after a long domestication history started off in Centro Mexico, it was developed into the range of present forms (González et al., 2011).

1.1.2. Production

Tomatoes are widely consumed around the world as fresh or processed products (Domínguez et al., 2016). They are the most consumed fresh vegetable in Europe, and the fourth most consumed in the United States (Castagna et al., 2013). The global production of tomatoes was around 182 million tonnes in 2018, with the leading producers being China (33.8%), following by India, the USA, Turkey, Egypt, Iran, Italy, Spain, Mexico, and Brazil (FAOSTAT, 2020). In 2018, tomato production in Europe was 13% of global amount; the UK produced 66,000 tonnes (Figure 1.1) (FAOSTAT, 2020). The tomato in UK is mainly imported from Spain and the Netherlands, and the import amount in 2017 was 374,000 tonnes (FAOSTAT, 2020; Heuvelink, 2018; Løvdal et al., 2019).

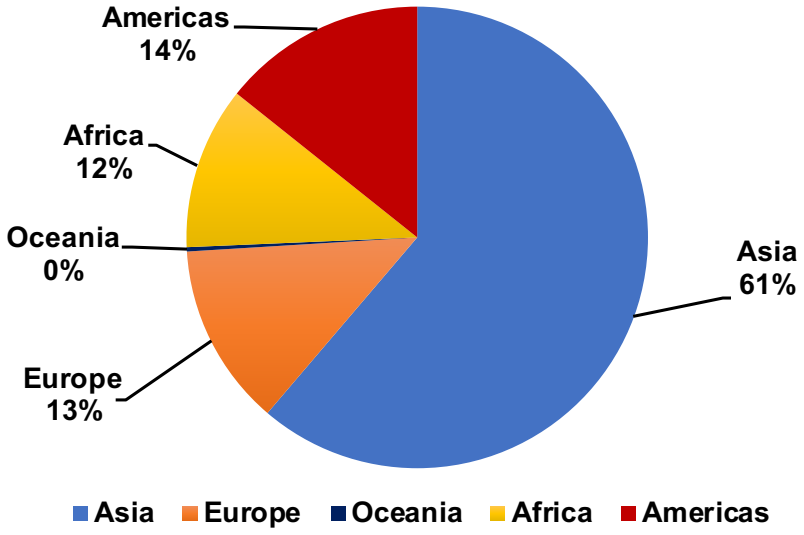


Figure 1.1 **Production of tomatoes in different regions of the world in 2018.** (Data from FAOSTAT, 2020).

Glasshouses are widely used for cherry tomato production to achieve a year-round cultivation system (Løvdal et al., 2019). The yields of some cherry tomato varieties such as Marasca, Brillantino, Ovalino, and Piccolo can be between 54 and 88 t/ha (Ceballos Aguirre and Vallejo Cabrera, 2012; Macua et al., 2008).

After harvesting, cherry tomatoes need to go through several steps, including packaging, quality assurance, and transportation, to arrive at retail stores (Norton and Fearne, 2009). Depending on the distance, it takes days to weeks for cherry tomatoes to arrive at retails. For example, it takes at least one week to import cherry tomatoes from Spain to UK (Norton and Fearne, 2009).

Packaging is one of the main reasons causing a high level of loss of tomatoes, as poor packing of warm tomatoes can result in condensation, fruit decay and the growth of bacteria and fungal mould (Løvdal et al., 2019; Murmu and Mishra, 2018). As a consequence, developing postharvest techniques are beneficial for the best possible utilization of cherry tomato and to reduce economic loss.

1.1.3. Development, ripening and structure of tomato

The development of tomato fruit begins from genetic regulation in the floral meristem, and continues to fruit growth, including cell division phase and cell expansion phase, till fruit ripening (Figure 1.2) (Quinet et al., 2019). The hormones involved in tomato fruit development during fruit set and growth are auxins, gibberellins, cytokinins and abscisic acid, while ethylene and auxins are the main hormones that control fruit ripening (Quinet et al., 2019).

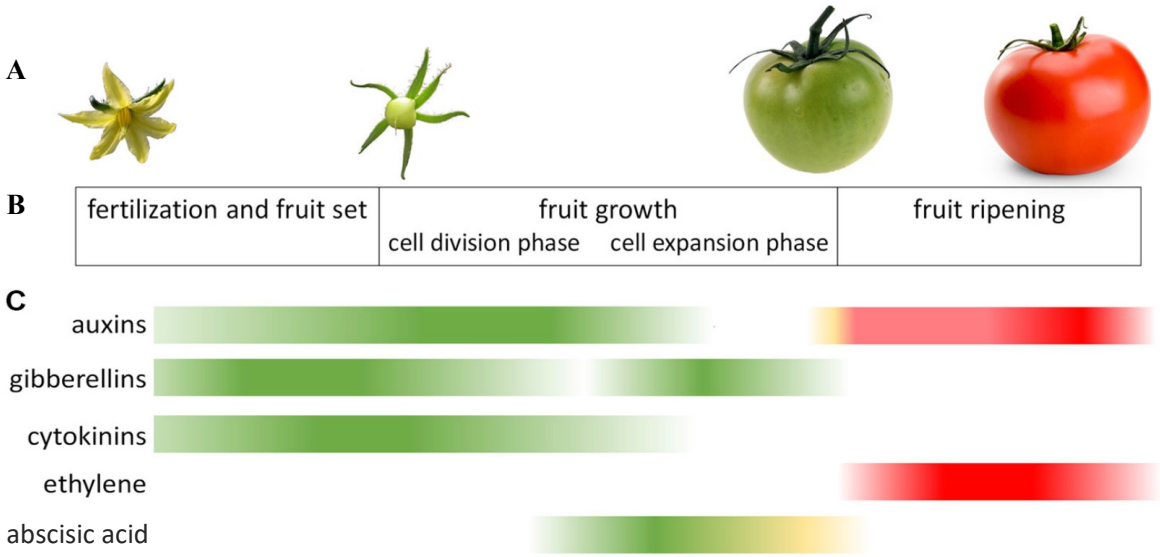


Figure 1.2 **Hormonal control of tomato fruit development.** (A and B) Main stages of tomato fruit development. (C) Main hormones involved in tomato fruit development during fruit set and fruit growth (green) and fruit ripening (red). (Image adapted from Quinet et al., 2019).

Fruit ripening is a complex process controlled by both external factors, such as light and temperature, and internal factors, such as hormone interplay and gene expression, leading to physiological and biochemical changes that determine colour, texture, flavour, aroma and nutritional quality (Mangaraj et al., 2019; Quinet et al., 2019; Valero and Serrano, 2010). There are different ripening processes and molecular mechanisms for climacteric and non-climacteric fruits (Gao et al., 2019). The ripening of climacteric fruit is triggered by an increase in ethylene production and respiration rate, but little ethylene is produced by non-climacteric fruits during ripening (Domínguez et al., 2016; Hasan et al., 2017). The biosynthesis and perception of ethylene are highly regulated by genes, involving *ACS2*, *ACO1* (ethylene biosynthesis genes) and a set of transcriptional factors (Figure 1.3) (Gao et al., 2019; Quinet et al., 2019).

As a climacteric fruit, tomato ripening requires three transcription factors (TFs): ripening inhibitor (RIN), colourless non-ripening (CNR), and non-ripening (NOR), and they modulate ethylene biosynthesis and signal transduction during fruit ripening (Gao et al., 2019; Quinet et al., 2019). Among the three TFs, RIN is the best studied regulator of tomato fruit ripening, which directly controls the expression of target genes involved in ripening-related events (Figure 1.3) (Qin et al., 2012; Quinet et al., 2019). RIN can directly bind to the promoters of tomato ripening genes, including *p*olygalacturonase2a (*PG2a*) and *expansan 1* (*EXP1*) (cell wall softening genes), *lipoxygenase* (*LoxC*) (aroma biosynthesis gene) and *phytoene synthase 1* (*PSY1*) (a gene that regulates lycopene biosynthesis) (Gao et al., 2019).

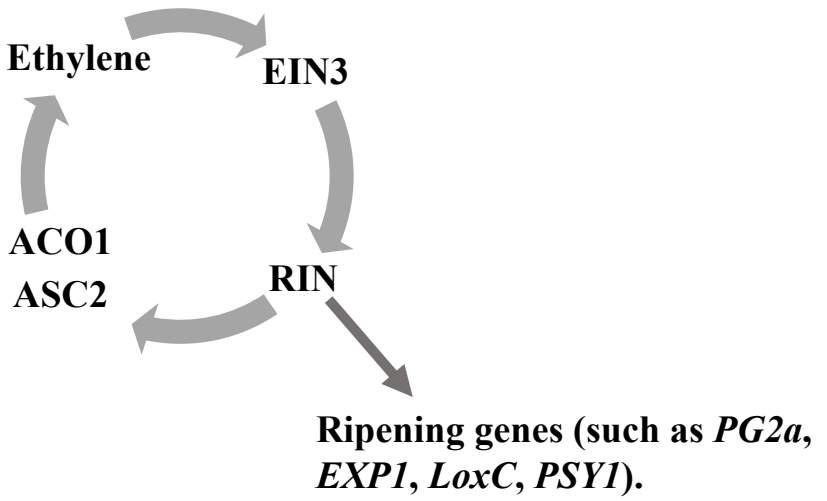


Figure 1.3 **MADS-loop in tomato.** Ethylene transcription factor EIN3 activates the *RIN*, while *RIN* activates ethylene biosynthesis genes 1-aminocyclopropane-1-carboxylic acid oxidase *1* (*ACO1*) and 1-aminocyclopropane-1-carboxylate synthase 2 (*ASC2*) forming a positive feedback circuit that results in autocatalytic ethylene production during ripening. RIN can also activate downstream ripening genes (such as *PG2a*, *EXP1*, *LoxC*, *PSY1*) (Image adapted from Gao et al., 2019).

The major tissues of tomato fruit are total pericarp, septum, locular tissue, placenta, columella and seeds, and the cell types of the pericarp include outer epiderimis, collenchyma, parenchyma, vascular tissue and inner epidermis (Figure 1.4) (Shinozaki et al., 2018). The colour change during fruit ripening is observed in the pericarp (Shinozaki et al., 2018).

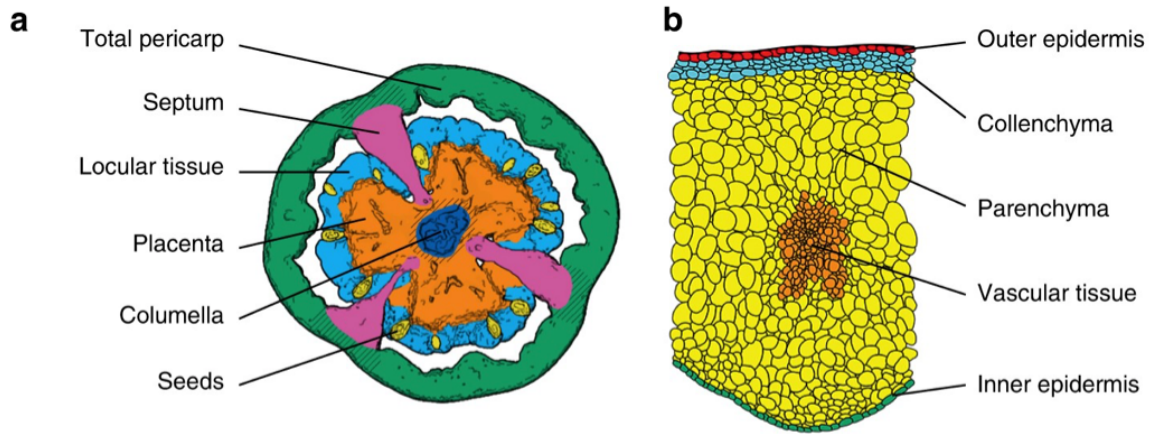


Figure 1.4 **A tissue/cell-based structure of tomato.** (a) Traced image of fruit tissues. (b) Traced image of pericarp cells (Shinozaki et al., 2018).

1.1.4. Ripening classification

To describe the ripeness of tomato, a traditional classification system, mainly based on external colour, has been developed by United States Department of Agriculture (USDA 2002), which classifies tomatoes into six ripeness stages (Figure 1.5):

1. mature green (the surface is 100% green in colour)
2. breaker (a noticeable break in colour with less than 10% of colour)
3. turning (10 - 30% red colour)
4. pink (30 - 60% red colour)
5. light red (60 -90% red colour)
6. red (more than 90% red) (Panjai et al., 2017).

Alternatives to the five-stage classification system exist in which the breaker stage is removed:

1. mature green (the surface is 100% green in colour)
2. turning (1- 25% red colour)
3. pink (25 - 50% red colour)
4. light red (50 - 75% red colour)
5. red (75 - 100% red colour) (Kou et al., 2016; Løvdal et al., 2019).

The colour change during ripening is mainly caused by a change in the relative chlorophyll (green) and lycopene (red) content (Liu et al., 2009). At the mature green stage, chlorophyll is the predominant pigment, and the ratio of chlorophyll and carotenoid is about 10:1 (Heuvelink, 2018). As the fruit ripens, there is an accumulation of carotenoid and a destruction of chlorophyll which ultimately reaches zero at the red-ripe stage (Heuvelink, 2018).

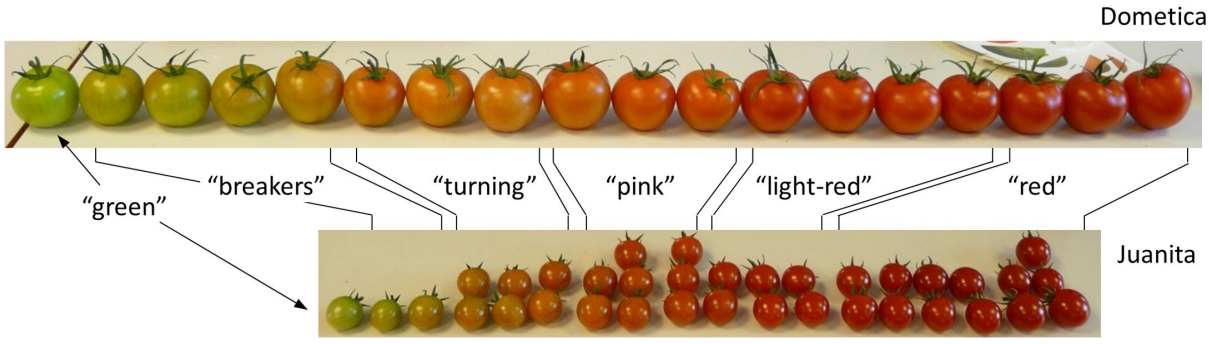


Figure 1.5 Fruit colour classification during ripening in tomato fruits of the 'Dometica' and 'Juanita' varieties (Ciaccheri et al., 2018).

1.1.5. Nutritional quality

Tomato quality can be divided into sensory quality, flavour quality and nutritional quality (Liu et al., 2018). Sensory quality comprises appearance, colour, as well as firmness that affects mouth feel, shelf life, wastage and transportation (Liu et al., 2018; Yang et al., 2017). Flavour quality depends on two vital factors, 'fruitiness' and 'sweetness', which are determined by three major components, sugars, acids and volatiles (Liu et al., 2018; Majidi et al., 2014). Glucose and fructose are the two major sugars that contribute to sweetness, and citric and malic acid contribute to sour taste in cherry tomatoes (Majidi et al., 2014; Petro-Turza, 1986). Nutritional quality is mainly contributed by ascorbic acid, carotenoids and phenolics (Dhakal and Baek, 2014b; Liu et al., 2018). Cherry tomato is the number one source of lycopene in the human diet, as well as an important source of β-carotene and ascorbic acid (Fagundes et al., 2015).

1.1.5.1. Ascorbic acid

Ascorbic acid, also known as vitamin C, is an important antioxidant in cherry tomato (Ioannidi et al., 2009; Ma et al., 2014). In plants, it acts as a cofactor for diverse enzymes, which contributes to the regulation of cell growth, division and expansion (Smirnoff and Wheeler, 2000). It also plays a crucial role in controlling plant growth, flowering time, and the onset of senescence (Davey et al., 2000; Ioannidi et al., 2009). As a signalling factor, ascorbic acid can participate in interactions with the environment, including pathogens and water availability (Fotopoulos et al., 2008; Ioannidi et al., 2009).

Depending on climatic conditions, genotype, stage of fruit development and ripening, and storage time, the content of ascorbic acid in cherry tomato fruits can vary substantially (Raiola et al., 2014). Abushita et al. (2000) reported that ascorbic acid content was between 15 and 21 mg/100 g FW in salad tomatoes grown in field. Whereas Yahia et al. (2001) found

that the content of ascorbic acid in glasshouse grown tomatoes in controlled hydroponic conditions could reach a maximum level of 94.9 mg/100 g at day 74 after fruit set, and then reduced slowly with the colour change.

As humans, and other primates, cannot synthesize ascorbic acid *de novo* due to a lack of the enzyme L-gulonolactone oxidase (an enzyme that produces vitamin C), or store it in the body, fresh fruits and vegetables have therefore become important sources of ascorbic acid (Ma et al., 2014; Naidu, 2003). The US recommended dietary allowances (RDA) of ascorbic acid are 90 mg/day for men and 75 mg/day for women (Frei and Traber, 2001; Naidu, 2003). As ascorbic acid is sensitive to light, air, heat and can be destroyed easily by long storage time and during the over processing of food, a minimum of 400 g of fresh vegetables and fruits per day is recommended by FAO/WHO (Naidu, 2003; WHO, 2003).

Adequate ascorbic acid intake has been proved to be related to reduced incidence of some diseases, including stroke and heart disease, whereas insufficient intake results in the disease of scurvy, which shows symptoms like dry skin, weariness, depression and open sores on the skin (Carr and Frei, 1999; Naidu, 2003; Raiola et al., 2014). It is widely known that ascorbic acid intake can prevent common cold infection by boosting immunity, or relieve symptoms and reduce the duration of the symptoms (Naidu, 2003). Ascorbic acid has also been reported to neutralize free radicals before they damage DNA and initiate tumour growth to prevent cancer, and it may also help the body's own free radicals by acting as a pro-oxidant to destroy tumours in the early stages (Block, 1991; Raiola et al., 2014).

1.1.5.2. Carotenoids

Carotenoids are lipid-soluble 40-carbon isoprenoids that are synthesized via the general isoprenoid synthetic pathway in chromoplasts of fruits and flowers, and in chloroplasts of green tissues in plants, but not in animals, making fruits and vegetables the major sources of

carotenoids for human (Liu et al., 2015; Rao and Rao, 2007; Ronen et al., 2000). Carotenoids take part in many physiological processes in plants, including growth, development, and response to environmental stimuli, and they also play important parts in photosynthesis machinery by carrying out the function of light harvesting where beta-carotene is in the photosystem 1 and 2 core light harvesting complexes (Rao and Rao, 2007; Ronen et al., 2000). In higher plants, carotenoids are responsible for most of the red, orange and yellow colours of flowers and fruits, attracting animals for pollination of flowers and the subsequent distribution of seeds from the fruits which develop (Ronen et al., 2000).

The carotenoid biosynthesis pathway is illustrated in Figure 1.6. Tomato has been used as a model system to study the carotenoid biosynthesis regulation, as the colour changes dramatically during fruit ripening (Ronen et al., 2000). At the early mature green stage of tomato fruits, the major carotenoids are lutein, β-carotene and violaxanthin, which is the same as in other green tissues, such as leaves (Ronen et al., 2000). Lycopene begins to accumulate at the breaker stage and the concentration increases 500-fold in ripe fruits, with a level of 70 μg/g fresh weight (FW) (Fraser et al., 1994; Ronen et al., 2000). In ripe red tomato fruits, the major carotenoids are lycopene (around 90%, red pigment), β-carotene (5%-10%, orange pigment), and lutein (1%-5%, yellow pigment) (Schofield and Paliyath, 2005).

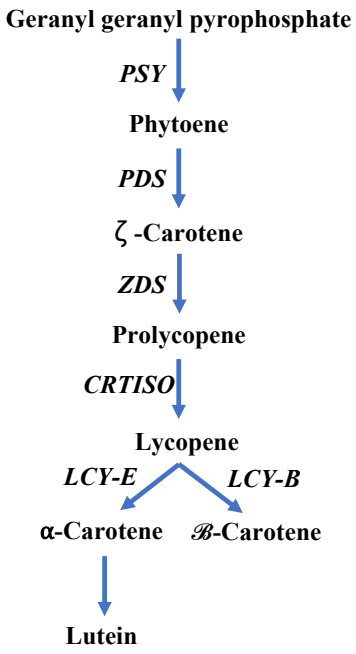


Figure 1.6 **Carotenoid biosynthesis pathway (Pandurangaiah et al., 2016).** *PSY*, *phytoene synthase*; *PDS*, *phytoene desaturase*; *ZDS*, *ζ-carotene desaturase*; *CRTISO, carotenoid isomerase*; *LCY-E*, *lycopene ε-cyclase*; *LCY-B, lycopene β-cyclase*.

The content of carotenoid in tomato fruits greatly influences customers' perception on them and their economic value as consumers prefer tomatoes with intense red colour (Schofield and Paliyath, 2005). The health benefits of carotenoids have been claimed to reduce the incidence of some human diseases, including cardiovascular diseases and cancer (Paiva and Russell, 1999; Rao and Rao, 2007). It has been reported that carotenoid intake is the most important nutritional contribution of tomato (Raiola et al., 2014).

Lycopene is an important member of the carotenoid family, and also an intermediate in biosynthesis of many other carotenoids (Figure 1.6) (Løvdal et al., 2019; Pandurangaiah et al., 2016). As a red pigment, lycopene can be found in some fruits and vegetables including tomato, chili pepper, watermelon and carrot, with tomato being the major source (Fadupin et al., 2012; Løvdal et al., 2019). Lycopene is the most abundant carotenoid in ripe red tomato

fruits, constituting about 80-90% of total carotenoids (Liu et al., 2011). The content can range from 0.03 to 27.0 mg/100 g FW in different varieties of tomato, with the highest content of 40 mg/100 g FW found in some wild species, such as *S. pimpinellifolium* (Adalid et al., 2010; Løvdal et al., 2019). Depending on the growing environment and cultivation method, lycopene content in cherry tomato can range from 1.6 to 14.6 mg/100 g FW (Løvdal et al., 2019). The changes between maturity stage, fruit colour and lycopene content as shown in Figure 1.7.

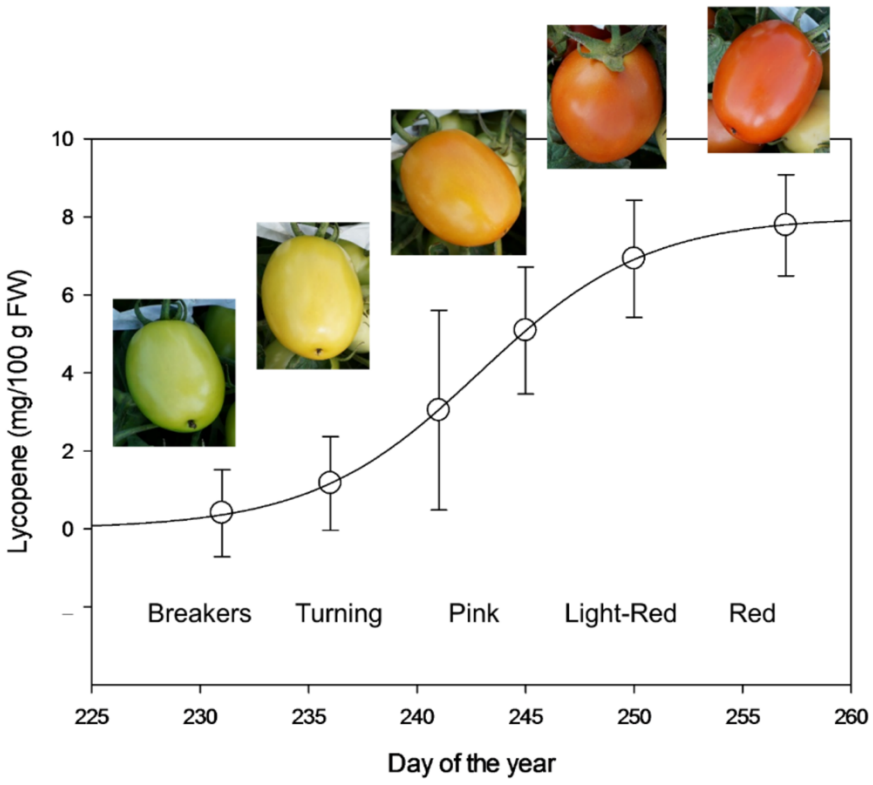


Figure 1.7 Lycopene concentration in processing tomatoes (cv. Calista) (Løvdal et al., 2019).

As a powerful antioxidant, lycopene provides protection against oxidation damage and can therefore reduce the risk of some cancers (Ansari and Gupta, 2004; González-Vallinas et al.,

2013; Raiola et al., 2014). It has also been shown to provide some protection from cardiovascular disease and osteoporosis (Raiola et al., 2014).

The recommended daily intake level of lycopene is 0.7 mg/day although different countries have different recommendations, for example, Finland 1.3 mg/day, UK and Germany 1.1 mg/day (Rao, 2002). Higher amounts have been suggested to combat oxidative stress and protect against chronic diseases, with a daily intake level of 5 - 7 mg recommended for healthy people, and higher level of 35 - 75 mg required for people suffering from cancer or cardiovascular diseases (Heath et al., 2006; Rao and Shen, 2002).

β-carotene is a precursor of vitamin A and responsible for the orange colour of tomato (Schofield and Paliyath, 2005). The content of β-carotene in field-grown tomatoes is 0.28 - 1 mg/100 g FW, and in commercial cherry tomatoes, the content can reach 1.2 mg/100 g FW depending on varieties (Barba et al., 2006; Frusciante et al., 2007). Similarly to lycopene, β-carotene is a strong antioxidant, and can provide photoprotection for humans by preventing photooxidative damage and sunburn (Raiola et al., 2014). It can also inhibit atherosclerosis and prevent myocardial infarction (Karppi et al., 2013; Stahl and Sies, 2003).

Lutein, a yellow carotenoid, is synthesized in chloroplasts and chromoplasts in plants (Giorio et al., 2013). The average content of lutein in tomato fruit is around 32 ug/100 g FW, while in varieties, such as Pera and Rambo tomatoes, it can reach up to 800 ug/100 g FW (Guil-Guerrero and Rebolloso-Fuentes, 2009; Perry et al., 2009). The consumption of lutein can prevent the development of atherosclerosis by reducing the inflammation and oxidative stress in the artery wall (Raiola et al., 2014). Lutein also plays an important part in protecting human against cardiovascular diseases and maintaining eye health when associated with

zeaxanthin, as lutein and zeaxanthin can protect eyes from harmful high-energy light waves, such as ultraviolet rays in sunlight (Giorio et al., 2013; Riccioni et al., 2012).

1.1.5.3. Total phenolics

Phenolic compounds are composed of an aromatic ring and one or more hydroxyl substituents (Balasundram et al., 2006; Raiola et al., 2014). As one of the most widespread classes of phytochemicals and the most important secondary metabolites in plants, phenolic compounds play important roles in physiological and morphological processes, including plant growth and reproduction (Balasundram et al., 2006; Bravo, 1998). Phenolic compounds contribute to sensory quality of vegetables and fruits, such as aroma, colour, and bitterness, and they can also protect against predators and pathogens (Alasalvar et al., 2001; Balasundram et al., 2006). Depending on genotype, growing environment and storage condition, the content of phenolic compounds in tomato fruits can vary largely (Raiola et al., 2014). According to Martínez-Valverde et al. (2002), the content in tomato fruits ranges from 25.9 to 50.0 mg/100 g FW depending on varieties.

The health benefits of phenolic compounds are mainly attributed to their antioxidant activity (Heim et al., 2002). Phenolics can regulate cellular signalling processes or directly serve as signalling agents during inflammation to protect against some diseases, including cardiovascular diseases, neurodegenerative diseases, obesity and type II diabetes (Aggarwal and Shishodia, 2004; Rahman et al., 2006; Raiola et al., 2014). They also play important roles in protecting against different types of cancers by interfering with the initial, promotional and progressive period (Marti et al., 2018).

In tomato fruits, the main phenolic compounds contributing to fruit quality are flavonoids, and they are the largest group of phenolic compounds and contribute to fruit colour,

fragrance, and aroma (Bertin and Genard, 2018; Raiola et al., 2014). The biosynthesis of flavonoids is shown in Figure 1.8. The content of flavonoids in different tomato types ranges from 1 to 30 mg/100 g FW (Slimestad and Verheul, 2009). The main flavonoids in tomato are Naringenin (45%), quercetin (39%), myricetin (10%) and kaempferol (5%) (Bertin and Genard, 2018; Slimestad and Verheul, 2009).

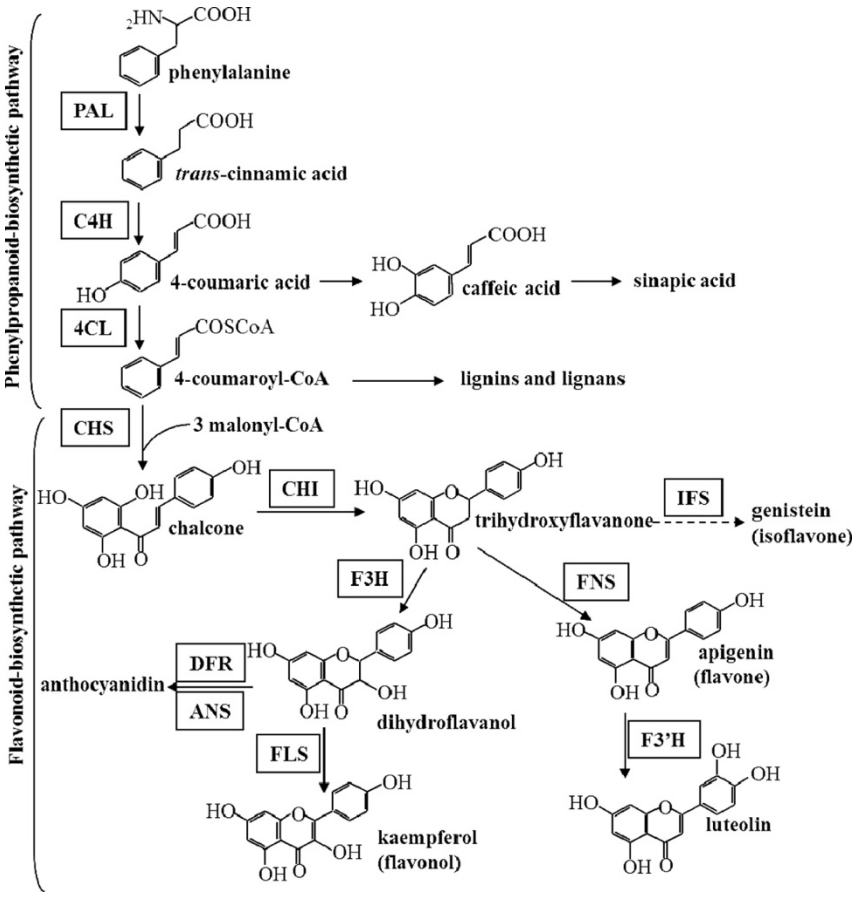


Figure 1.8 **Biosynthetic pathway of flavonoids.** Biosynthetic pathway of flavonoids consists of phenylpropanoid- and flavonoid-biosynthetic pathways, and is catalysed by the following enzymes: PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FNS, flavone synthase; F3H, flavonoid 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanin synthase; F3′H, flavonoid 3′-hydroxylase; and IFS, isoflavone synthase (Kanazawa et al., 2012).

1.1.6. Postharvest techniques

According to the FAO/WHO report (2003), to prevent chronic diseases such as diabetes, obesity and cardiovascular diseases, a minimum daily intake of 400 g of fruits and vegetables (except potatoes and other starchy tubers) is recommended. However, the consumption of vegetables and fruits in European countries is typically far less than this (Løvdal et al., 2019). Therefore, the consumption of vegetables and fruits with higher nutritional quality might be an alternative option to obtain more nutritional components and reduce the incidence of chronic diseases and cancer.

Cherry tomatoes are highly perishable and undergo physiochemical changes, such as softening, colour evolution, and aroma development during ripening, and a subsequently loss of quality (Domínguez et al., 2016; Ioannidi et al., 2009; Mangaraj et al., 2019). To extend shelf-life, preserve quality and maintain market value of vegetables and fruits, many postharvest techniques, including physical, chemical and gaseous treatments, have been developed (Fagundes et al., 2015).

1.1.6.1. Temperature

Temperature control is one of the most common and effective postharvest treatments to maintain quality and prolong shelf life of vegetables and fruits due to its dramatic effect on the rate of biochemical, physiological and microbiological reactions (Ayala-Zavala et al., 2004; Liplap et al., 2013; Wu, 2010). Low-temperature storage (0-12°C) is frequently used to reduce respiration and metabolic activities of products, and inhibit microbiological development (Brasil and Siddiqui, 2018).

However, in tomatoes, low temperature can cause chilling injuries that result in poor colour development, uneven ripening, surface pitting, increased water-soaked areas, loss of aroma

volatiles, and disease and fungal infection susceptibility (Mangaraj et al., 2019; Park et al., 2018b). Tomatoes are sensitive to chilling injury and symptoms can develop easily if they are transferred to ambient temperature, such as 20°C, from low-temperature storage, such as 12°C (Lurie and Sabehat, 1997; Park et al., 2018b). As a result, after cold transportation or cold storage, tomatoes are very susceptible to chilling injury when moved to warmer areas, such as retail stores (Park et al., 2018b).

To reduce chilling injury symptoms, tomatoes must be stored at appropriate temperatures. According to Cantwell (2000), tomatoes are prone to damage when held at temperatures below 10°C for more than two weeks, or below 5°C for more than six to eight days. The optimum storage temperature for tomatoes at the mature green stage is 12.5 – 15°C, which can preserve them for 14 days without a large reduction of sensory quality (Cantwell, 2000; Park et al., 2018b). The optimum storage temperature is lower for tomatoes at the light red stage, which is 10 – 12.5°C (Cantwell, 2000). As shown in Figure 1.9, a temperature of 10 – 16°C is recommended for the transit and storage of breaker stage tomatoes, and a temperature of 18 – 21°C is recommended for tomato ripening (Cantwell, 2000).

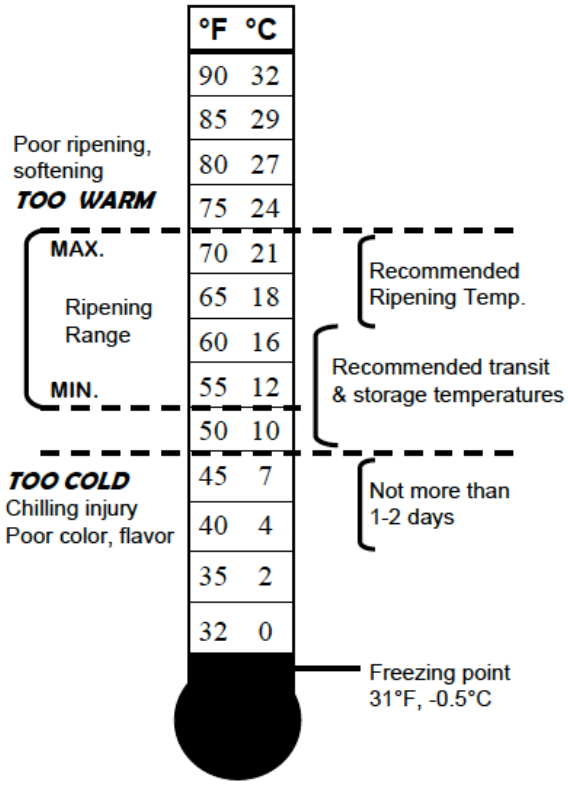


Figure 1.9 Tomato storage at different temperatures (Cantwell, 2000).

Modified atmosphere packaging (MAP) can be used to reduce chilling injury symptoms for chilling-sensitive fruit during cold storage (Park et al., 2018b; Pereira et al., 2004; Porat et al., 2004; Rana et al., 2015). According to Park et al. (2018b), MAP with 14.9–16.7% O_2 and 4.2–7.3% CO_2 reduced chilling injury symptoms, such as surface pitting, uneven ripening or failure to ripen of tomato effectively during cold storage (4°C).

1.1.6.2. Light irradiation

Light is one of the most important factors that controls plant growth and development, including flowering time, morphogenesis and photosynthesis, but plants cannot benefit from the entire spectrum of light (Hasan et al., 2017; Ma et al., 2014). There are two major photoreceptors, phytochromes and cryptochromes, that perceive light to control morphogenetic and photosynthetic responses in plants (Hasan et al., 2017). Phytochromes are

responsible for the absorbance of red and far-red light, whereas cryptochrome photoreceptors can absorb blue light and ultraviolet A (UV-A) light (Bohne and Linden, 2002; Hasan et al., 2017; Ma et al., 2014).

Traditional lighting systems, such as fluorescent, xenon and incandescent lamps, have a broad spectrum of wavelengths, and the use of them may generate excessive heat, which will affect plant growth and development (Hasan et al., 2017). Plants may also develop inadequate protective mechanisms to protect themselves against UV or infrared radiations under these lighting systems, which can cause undesirable effects on plant growth and development (Hasan et al., 2017; Mitchell et al., 2012; Morrow, 2008).

Light-emitting diodes (LEDs) are one of the most widely used light sources that can emit narrow spectrum radiation with minimal heat effects and high efficiency (Hasan et al., 2017; Mitchell et al., 2012). Compared to traditional lighting systems, LEDs have greater longevity, waste less energy, and have no thermal photon emission (Hasan et al., 2017; Morrow, 2008; Yeh and Chung, 2009).

LEDs have been applied to preharvest fruits and vegetables to promote plant biomass and nutrient content in glasshouse and growth chambers, and they can also induce disease resistance to a wide range of phytopathogens in standing crops (Brasil and Siddiqui, 2018; Hasan et al., 2017). It has been shown that the application of preharvest blue or red LEDs can significantly increase the yield and quality of vegetables and fruits, such as cucumber and strawberry, when compared with white or solar light (Choi et al., 2015b; Hao et al., 2012). In addition, several studies have also reported that single-spectral red or blue LEDs induced accumulation of primary and secondary metabolites, such as starch, soluble protein and

sugars, in plant when compared with white fluorescent (Johkan et al., 2010; Kim et al., 2013; Li et al., 2012; Li et al., 2010).

Blue light affects many aspects of plant growth and development including flowering, and it can also regulate many plant responses such as biomass production and stomatal opening (Gong et al., 2015; Ma et al., 2014; Xu et al., 2012). Blue light benefits chlorophylls, vitamin C, soluble sugar, and soluble protein accumulation in non-heading Chinese cabbage (Li et al., 2012). It has been reported that blue light could increase anthocyanin content and regulate the biosynthesis pathway of flavonoid in postharvest strawberries (Shi et al., 2014; Xu et al., 2014). According to Gong et al. (2015), blue light could maintain the content of total soluble solids, induce the decrease of total titratable acid, and induce fruit ripening through increasing ethylene biosynthesis in postharvest peaches.

Red and far-red light are detected by the same plant photoreceptor, phytochromes, and they can regulate plant development, increase flowering and budding, and enhance starch accumulation in plant (Alba et al., 2000a; Ma et al., 2014; Wu et al., 2007). It has been reported that red light could delay the yellowing process, reduce ethylene production, and delay senescence in postharvest broccoli (Ma et al., 2014). Lee et al. (2016) reported that red light induced the content of glucosinolates in postharvest Chinese cabbage and kale compared to dark condition, and when combined with blue light, it could increase the content of total polyphenols. It has been reported that red light increased carotenoid accumulation in cherry tomatoes, and the red-light-induced carotenoid accumulation was reversible by red/far-red light (Alba et al., 2000a; Liu et al., 2009).

1.1.6.3. Modified atmosphere packaging (MAP)

Another strategy to extend shelf life and control the postharvest degradation of vegetables and fruits is to use modified atmospheres, such as controlled atmosphere (CA) and modified atmosphere packaging (MAP) (Fagundes et al., 2015). In CA storage the atmosphere composition and concentration are tightly controlled on a continuous basis, while in MAP a specific gas mixture is flushed into packages once, but this will change automatically during storage (Choubert and Baccaunaud, 2006; Majidi et al., 2014).

It has been reported that CA with low O_2 and/or high CO_2 content can extend shelf life, reduce respiration rate and ethylene production, delay colour change and softening, and inhibit some diseases and physiological disorders of guavas (Singh and Pal, 2008; Teixeira et al., 2007; Teixeira and Durigan, 2010; Teixeira et al., 2016). However, prolonged CA storage leads to physiological disorders on vegetables and fruits, including the accumulation of acetaldehyde and ethanol, failure to ripen, loss of flavour and odour and damage caused by high CO_2 (Teixeira et al., 2016).

MAP is a widely used technique in the preservation of vegetables and fruits by modifying the air inside of the packages with a mixture of carbon dioxide, oxygen, and nitrogen actively (active MAP) or passively (passive MAP) (Guo et al., 2019; Ye et al., 2012). Active MAP is modifying the atmosphere composition in the package by replacing air with a mixture of desired gases, while passive MAP is sealing product with selected polymeric films, and a desired gaseous environment is generated naturally due to product respiration and gas exchange through the packaging material (Choi et al., 2015b; Ye et al., 2012). Cherry tomatoes treated with active MAP showed inhibited senescence, reduced respiration, and inhibited chemical reactions that alter compounds such as organic acid, sugars, and lycopene

(Fagundes et al., 2015). Compared with active MAP, passive MAP is easier to achieve by introducing different numbers and dimensions of micro-perforations on packaging films (Elwan et al., 2015). Passive MAP has been reported to extend shelf-life of sugar snap peas for three weeks by reducing metabolic activities and mechanical damage (Elwan et al., 2015). Few applications of passive MAP on cherry tomatoes have been reported although it has been found that passive MAP could preserve freshness and firmness, inhibit weight loss, and reduce degradation rate of sugars and organic acids (D'Aquino et al., 2016).

The optimal concentration of O_2 and CO_2 depends on the nature of produce (Domínguez et al., 2016). Generally, the combination of 3-8% CO_2 and 2-5% O_2 are recommended for the storage of vegetables and fruits (Fagundes et al., 2015; Farber, 1991). The best gas combination for tomatoes is 3-5% O_2 and 0-5% CO_2 (Fagundes et al., 2015; Nunes et al., 1996; Sandhya, 2010).

1.1.6.4. Others

There are many other postharvest techniques being researched or used including chemical (antioxidants, antimicrobials and anti-browning), physical (heat and edible coatings), and gaseous (ozone, ethylene and 1-Methylcyclopropene) treatments (Fagundes et al., 2015; Mahajan et al., 2014).

Chemical-based antimicrobial and anti-browning agents, including chlorine-based solutions, peroxyacetic acid (PAA), organic acids and hydrogen peroxide (H_2O_2), offer the possibility to maintain the safety and quality of postharvest vegetables and fruits (Artés et al., 2009; Mahajan et al., 2014). The application of H_2O_2 on fruits, such as apples, melons and tomatoes, extends the shelf life, and reduces pathogen population (Cengiz and Certel, 2014;

Mahajan et al., 2014). But there are factors, such as internalization of bacteria, that restrict the efficacy of these chemical agents (Mahajan et al., 2014).

Heat treatments, including hot water or air, and vapor heat, have been used as an alternative to chemical treatments for postharvest vegetables and fruits, and can control fungal pathogens and insects, delay ripening, maintain fruit quality, and modify fruit response to cold stress (Lurie, 1998; Mahajan et al., 2014). But improper temperature (above 63°C) of heat treatment may cause external damage, such as peel browning, pitting and yellowing of green vegetables, and internal defects, such as tissue damage (Lurie and Pedreschi, 2014; Tang, 2007).

Edible coatings are thin coating layers, such as mineral oils, polyvinyl acetate and *Aloe vera*, applied to the surface of fresh products to provide a partial barrier (Dhall, 2013; Gol et al., 2013; Mahajan et al., 2014). The application of edible coatings can reduce respiration, slow down senescence, preserve texture, and protect the products against mechanical damage (Dhall, 2013; Mahajan et al., 2014; Mohebbi et al., 2012). However, the cost of film or coating production equipment and the lack of edible materials has limited its application at the industrial level (Mahajan et al., 2014).

As an inhibitor of ethylene perception, 1-Methylcyclopropene (1-MCP) has been used to control ethylene responses of horticultural products (Blankenship and Dole, 2003; Mahajan et al., 2014). The application of 1-MCP on fruits and vegetables, such as cucumber, apple, banana, peach and tomato, has been approved in more than 50 countries (Mahajan et al., 2014). It has been reported that 1-MCP affects ripening and the senescence process, flavour,

fruit softening, and nutritional quality, but cultivar, genotype and maturity effects can be highly variable (Huber, 2008; Mahajan et al., 2014; Watkins, 2006).

1.2. Project aims and objectives

This research aimed to investigate the effect of postharvest techniques, including temperature, light irradiation and MAP treatment on the nutritional quality of cherry tomatoes, and to determine the best preservation method to use for cherry tomatoes during storage.

The main objectives of this research were:

- To investigate effects of temperature on nutritional quality in cherry tomatoes, and to find out the optimum temperature for the following experiments.
- To investigate effects of postharvest blue and red/far-red light and their irradiation time on nutritional quality in cherry tomatoes.
- To develop a combined method of red/far-red light and MAP to preserve nutritional quality of cherry tomatoes during storage.
- To explore the role and mechanism of red/far-red light in the regulation of lycopene biosynthesis.

2. Methods

2.1. Plant materials and treatments

2.1.1. Cherry tomatoes

Cherry tomatoes (*Solanum. lycopersicum* var. *cerasiforme*) (cv. Piccolo) were harvested at mature green stage from a commercial greenhouse in Worcestershire, UK. Fruits were transported in darkness in a foam box to the laboratory within one hour, and then separated from the vines. Mature green fruits with uniform size and shape, as well as absence of any injury were selected, and then hand washed with tap water and air-dried at room temperature (Figure 2.1). When dry, fruits were evenly placed onto plastic trays without touching each other, and then transferred to controlled environment cabinets (970, Sanyo) with a constant relative humidity of 85%.

Figure 2.1 Cherry tomatoes harvested from glasshouse.

2.1.2. Temperature treatments

In this experiment, three temperatures (5, 15 and 20°C) were chosen to represent chilling injury sensitive, storage suitable and ripening suitable temperature, respectively. The temperatures and constant relative humidity (85%) were set up in controlled environment cabinets in advance and lasted for 28 days.

Cherry tomatoes were divided into three groups for different temperature treatments, and they were stored in the cabinet for 28 days until the appearance of fruits was unacceptable to the consumer (shrinking surface, bitter smell, and soft when given a light squeeze). There were 25 fruits for each replicate and three replicates for each treatment. Two fruits were chosen randomly from each replicate at the beginning of the storage (day 1), and three fruits were collected at day 5, 10, 15, 20, 25, 28 from each replicate of each treatment. Fruits were frozen immediately with liquid nitrogen and then freeze dried. Dried samples were ground into powder and stored in screw-cap tubes covered with aluminium foil, and then kept at -80°C for the analysis of ascorbic acid, lycopene, β-carotene, lutein, total phenolics, and flavonoids. The remaining fruits were used to assess appearance and weight loss.

2.1.3. Light treatment

Red/far-red and blue light, temperatures (15°C) and constant relative humidity (85%) were set up in controlled environment cabinets (970, Sanyo) in advance and lasted for days. Fruits were divided randomly for different treatments, and they were stored in the cabinet for 33 days until the appearance of fruits was unacceptable to the consumer (shrinking surface, bitter smell, and soft when given a light squeeze).

Treatments:

(1) Control: complete darkness.

(2) Short-duration red/far-red light irradiation (R/FR-S): 5 days under continuous red/far-red light irradiation, and then transferred to darkness for 28 days.

(3) Middle-duration red/far-red light irradiation (R/FR-M): 15 days under continuous red/far-red light irradiation, and then transferred to darkness for 18 days.

(4) Long-duration red/far-red light irradiation (R/FR-L): 33 days under continuous red/far-red light irradiation.

(5) Short-duration blue light irradiation (B-S): 5 days under continuous blue light irradiation, and then transferred to darkness for 28 days.

(6) Middle-duration blue light irradiation (B-M): 15 days under continuous blue light irradiation, and then transferred to darkness for 18 days.

(7) Long-duration blue light irradiation (B-L): 33 days under continuous blue light irradiation.

Figure 2.2 Cherry tomatoes treated with blue light (left) or red/far-red light (right).

The red/far-red and blue light were provided by red and blue light filters covering on white tubes (TL-D Eco 32W/840, Philips), and the lamp tubes were installed parallel to each other on the top of cabinets. Fruits were aligned parallel to the light tubes at equal distance, and the fruit lines were shorter than the length of the lamp tubes to ensure the uniformity of irradiation intensity for all samples. The holding trays were covered with aluminium foil to avoid light reflection. The red/far-red and blue light intensity were 10.1 μmol m^{-2} s^{-1} and 21 μmol m^{-2} s^{-1}, respectively. The wavelength of the red/far-red light through the red filter was between 607-718 nm, with the peak at 707 nm. The ratio of red and far-red light was 0.89, calculated using the area under the graphed line between 600-700 nm for red light, and between 700-750 nm for far-red light, measured with ImageJ software. The wavelength of the

blue light was between 425-500 nm, with the peak at 430 nm. The light intensity and spectrum were measured at the three-quarter fruit height level using a light spectroradiometer (EPP 2000, StellarNet Inc, USA) at the beginning of the experiment.

There were 28 fruits for each replicate and three replicates for each treatment and control. Two fruits were chosen randomly from each replicate at the beginning of the storage (day 1). Three fruits were collected at day 5, 10, 15, 20, 25, 28, 33 from each replicate of every treatment and control. Fruits were frozen immediately with liquid nitrogen and then freeze dried. Dried samples were ground into powder and stored in screw-cap tubes covered with aluminium foil, and then kept at -80°C. These samples (168 samples) were used for the analysis of ascorbic acid, lycopene, β-carotene, lutein, total phenolics, and flavonoids. The remaining fruits from treatment R/FR-L, B-L and control were used to assess appearance and weight loss.

2.1.4. Passive MAP and light treatments

Fruits were placed in plastic trays (volume, 716 mL) and sealed with a polythene bag (length, 19.1 cm; width, 19.1 cm; thickness, 50 μm; O_2 transmission rate, 3.3×10^{-4} L m^{-2} s^{-1} MPa^{-1}; CO_2 transmission rate, 2.15×10^{-5} L m^{-2} s^{-1} MPa^{-1}). Each of these packages was perforated with 15 holes using a 0.45-mm-diameter needle. Perforations were done in three parallel lines on the top of the package, with lines distant from each other 2.6 cm, and holes from each other distant 2 cm in the same line. The O_2 and CO_2 permeance was measured by the method described in Murmu and Mishra (2017). Packages were divided randomly into two groups: one group was treated with white light (MAP), and the other was treated with red/far-red light (R/FR-MAP). Fruits placed in plastic trays without sealing with polythene bags were also divided into two groups: one group was treated with white light (Control), and the other was treated with red/far-red light (R/FR).

All packages were placed in controlled environment (970, Sanyo) with temperature of 15ºC and relative humidity of 85% for 30 days until the appearance of fruits was unacceptable to the consumers (shrinking surface, bitter smell, and soft when given a light squeeze). Packages of MAP treatment and control were placed at the same metal shelf of cabinet 1 (with white light irradiation) at equal distance to ensure the uniformity of irradiation intensity for all packages, and packages of treatment R/FR-MAP and R/FR were also placed at the same metal shelf of cabinet 2 (with red/far-red light irradiation) at equal distance (Figure 2.3). The white light was provided by white lamp tubes (TL-D Eco 32W/840, Philips), and the lamp tubes were installed parallel to each other on the top of cabinets. The white light intensity was 100 μmol m^{-2} s^{-1}. The setting up of red/far-red light was the same as the one above (See 2.1.3 for details). The light intensity and spectrum were measured at the three-quarter fruit height level at the beginning of the experiment using a light spectroradiometer (EPP 2000, StellarNet Inc, USA).

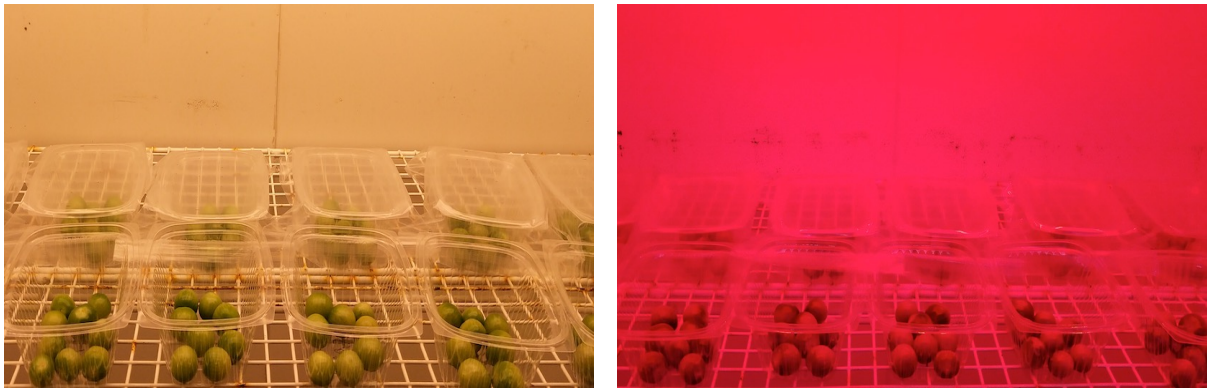


Figure 2.3 Wrapped and unwrapped cherry tomatoes treated with white (left) and red/far-red light irradiation (right).

Each package was filled with eight fruits and weighed approximately 88 g. The treatments and control each had 24 packages, and three packages of each was collected at each inspection time (day 1, 5, 10, 15, 20, 25, 30) to determine respiration activity and contents of

nutritional quality. The fruits used for nutritional quality measurement were frozen immediately with liquid nitrogen and freeze dried. Dried samples were then ground into powder and stored in screw-cap tubes covered with aluminium foil and stored at -80°C. And there were 84 samples used for the analysis of ascorbic acid, lycopene, β-carotene, lutein, total phenolics, and flavonoids. The remaining packages of each treatment and control was used to record appearance.

2.2. Appearance and weight loss

Fruit appearance, based on the depth of colour, was assessed at day 1, 5, 10, 15, 20 and 25 visually. Initial assessment (days 1, 5 and 10) was undertaken on three fruits per treatment which was increased to all 15 fruits at days 15, 20 and 25.

All fruits were weighed at day 1, 5, 10, 15, 20, 25. The weight loss was calculated and expressed as percent loss from initial weight.

2.3. Nutritional quality

2.3.1. Ascorbic acid analysis

The ascorbic acid content was determined with 2,6-dichloroinphenolindopehnol titration method described by Moo-Huchin et al. (2014).

0.15 g freeze-dried powder of fruit was homogenized in 5 mL of 2% (w/v) oxalic acid solution and then centrifuged at 8000 g for 10 min at 4°C (Fisherbrand, GT1) and filtered with filter paper. 1 mL of filtrated solution was diluted by 5 mL of 2% (w/v) oxalic acid solution and then titrated with 0.01% (v/v) of 2, 6-dichlorophenolindophenol. The final point was considered when the solution showed a very faint beige/pink colour for 30 s. The

ascorbic acid concentration was calculated according to the titration volume of 2, 6-dichloroinphenolindophenol and expressed as mg per g dry mass.

2.3.2. HPLC analysis of carotenoids

Extraction of carotenoids was carried out by the method of Moo-Huchin et al. (2014). 0.5 g freeze-dried powder was weighed and transferred into 50 mL centrifuged tubes covered with aluminium foil to avoid light. After adding 6 mL of 0.1% (v/v) butylated hydroxytoluene (BHT) in ethanol, tubes were heated in an 85°C water bath for 5 min, and 0.5 mL of 80% (v/v) KOH was added, and incubated again in 85°C water bath for 10 min. These tubes were immediately put in ice and added with 3 mL of cold HPLC water. After 3 mL of hexane (HPLC plus, >=95%) was added, these tubes were vortexed thoroughly and centrifuged at 3000 rpm for 3 min (Fisherbrand, GT1), and then the supernatant was transferred to a new tube. The hexane extraction was repeated a further two times to get a final volume of approximately 9 mL. The extracts were put in an evaporator until dry, and then re-suspended with 0.25 mL of absolute methanol (for HPLC) and 0.25 mL of 1,2-dichloroethane (HPLC, >=99.8%).

Carotenoids were determined with an Agilent 1100 series HPLC. 20 μL of sample was injected into chromatographic column C8 (4 um particle size, 4.6 mm x 250 mm) at a flow rate of 1 mL/min with mobile phase of methanol and HPLC water. Analytes were identified and calculated by comparison of their retention time with those of authentic standards (lycopene, β-carotene, and lutein). Results were represented as mg per 100 g dry mass.

2.3.3. Total phenolics and flavonoid analysis

Extraction of total phenolics and flavonoids was carried out according to the method described by Moo-Huchin et al. (2014).

The total phenolics content was measured using Folin-Ciocalteau's phenol reagent according to the method described previously by Yu et al. (2012) with some modifications. 1 mL of filtered solution or standard solution of gallic acid was mixed with 3 mL of distilled water and 1 mL of Folin-Ciocalteu's phenol reagent. After 8 min, 2 mL of 7.5% (w/v) Na_2CO_3 was added to the mixture followed by the addition of 3 mL of distilled water, and thoroughly mixed. After incubation for 30 min at 25°C, the absorbance was read at 765 nm using a spectrophotometer (JENWAY 6320D) against a blank which contained all reagents except the fruit extraction. Gallic acid was used as a standard, and total phenolic content was expressed as mg gallic acid equivalents per g dry mass.

The flavonoids content was determined using the colorimetric assay described by Toor and Savage (2005) with some modifications. 1 mL of extraction or standard solution of quercetin was diluted by 1 mL of distilled water. 0.4 mL of 5% (w/v) $NaNO_2$ was then added to the solution. After 5 min, 0.4 mL of 10% (w/v) $AlCl_3$ was added, and the mixture was allowed to react for 6 min before adding 2 mL of 4% (w/v) NaOH. The solution was then immediately diluted to a final volume of 5 mL with distilled water and thoroughly stirred. The absorbance was read at 510 nm using a spectrophotometer (JENWAY 6320D) against blank which contained all reagents except fruit extraction. Results was expressed as mg quercetin equivalents per g dry mass.

2.4.Gene expression

2.4.1. RNA extraction

Total RNA was isolated using the Monarch Total RNA Miniprep Kit (New England BioLabs Inc.) according to the manufacturer's protocol as below.

Cherry tomatoes were frozen with liquid nitrogen and ground into powder with a pestle and mortar. The tissue was collected into 2 ml Eppendorf tubes and mixed with 800 µL DNA/RNA protection reagent using a bead mill. The tubes were spun for 2 min at 16,000 x g in centrifuge (Sigma) to pellet debris, and then the supernatant was transferred to an RNase-free microfuge tube. An equal volume of RNA Lysis Buffer was then added and vortexed briefly. The samples were then transferred to a gDNA removal column (Monarch Total RNA Miniprep Kit) fitted with a collection tube to remove most of the genomic DNA. An equal volume of ethanol (≥ 95%) was then added to the flow-through and mixed thoroughly by pipetting. The mixtures were transferred to an RNA purification column (Monarch Total RNA Miniprep Kit) fitted with a collection tube and spun for 30 seconds in centrifuge (Sigma), and the flow-through was discard afterwards. DNase I treatment (Monarch Total RNA Miniprep Kit) was used to remove residual gDNA. 500 µL RNA Priming Buffer was added and spun for 30 s. The column matrix was washed twice with 500 µL RNA Wash Buffer, and then 50 µL of Nuclease-free Water added to elute the RNA. The RNA was kept at -80°C freezer for further experiment.

2.4.2. RNA quantification and qualification and cDNA synthesis

The quantity and quality of RNA were assessed with a Nanodrop spectrophotometer (Thermo Fisher Scientific) by measuring the absorbance ratio of A260/A280 and by electrophoresis on a 2% (w/v) agarose gel.

First-strand cDNA was synthesized using UltraScript 2.0 cDNA Synthesis Kit (PCR Biosystems Ltd., London, UK). 1µg of total RNA was mixed with 4 µL of cDNA Synthesis Mix and 1 µL of RTase. PCR grade dH_2O was then added to make a final volume of 20 µL. The mixture was incubated at 42°C for 30 min, and then at 85°C for 10 min to generate cDNA.

2.4.3. Quantitative RT-PCR analysis

For RT-qPCR analysis, 10 µL of PowerUp SYBR green PCR master Mix (Applied Biosystems), 7 µL of molecular H_2O, 1 µL of gene specific forward primer, 1 µL of gene specific reverse primer and 1 µL of cDNA were mixed to a final volume of 20 µL. 19 µL of samples were transferred to a qPCR plate, and each sample was run in triplicate. The primers used are shown in Table 2.1.

Quantification was performed with the QuantStudio Design and Analysis Software (version 1.5.0). Fruit collected at day 1 was used as calibration sample, and actin was used as internal reference gene. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 2.1 Genes and primers sequence used for RT-qPCR expression analysis.

Gene symbol	Name	Primer name	Sequence (5′ → 3′)
Actin[1]	Actin	*Actin*-F	TGTCCCTATCTACGAGGGTTATGC
		Actin-R	AGTTAAATCACGACCAGCAAGAT
PSY[2]	Phytoene synthase	*PSY*-F	GATGAGGCAGAGAAAGGCGT
		PSY-R	GCGGTACAAGACCAAAGATGC
ZDS[2]	Zeta carotene	*ZDS*-F	TCGGAGTGCCTGTTGTTACC
		ZDS-R	GCTCCAAGTCCTGCAACTCT
LCYb[2]	Chloroplast lycopene beta cyclase	*LCYb*-F	AAGAACGAATGGTGGCTCGT
		LCYb-R	ACCACCGATTCCAACGACTC
LCYe[2]	Chloroplast lycopene epsilon cyclase	*LCYe*-F	GCCGTGCCTATGGAAGAGTT
		LCYe-R	AAAACACCTGCCTCCACACA
PHYA[2]	Phytochrome A	*PHYA*-F	CTGGTTTTCCTGGGGCTCTT
		PHYA-R	CCTGAACCAGAACAGCCAGT
PHYB[2]	Phytochrome B	*PHYB*-F	CCACAGTTCAGCTCGGTTCA
		PHYB-R	TTTTCAGCCAACTGCGATGC

HY5[2]	Elongated hypocotyl 5	*HY5*-F	AGCGACGAGTTCTATTGCCG
		HY5-R	TCCGGCACTCTTCTGATCTC
PIF3[2]	Phytochrome-interacting factor 3	*PIF3*-F	AAGGGGTTCCGGTGGAGATA
		PIF3-R	TGTCTGATTCTGTGGGCAGC
ACS2[3]	1-aminocyclopropane-1-carboxylate synthase 2	*ACS2*-F	TGGATGATGGAACGGTTGATATTGC
		ACS2-R	CCATTGTTGCTTCTGTTCCATCGAAC
RIN[3]	Ripening inhibitor	*RIN*-F	TAGTCGTGGCAAGCTTTATGAAT
		RIN-R	TCTTGGTAGTTGTTCTGTGAATCTG

[1] Su et al. (2015), [2] Xie et al. (2019), [3] Yu et al. (2019)

2.5.Statistics analysis

SPSS Version 25.0 was used for statistical analysis of data. The significant difference between treatments was analysed by means of one-way ANOVA and Tukey's honestly significant difference (HSD) at a significance level of 0.05. Univariate analysis was conducted using SPSS to find the two-factor interaction effect of storage time and treatment.

3. Effects of temperature on nutritional quality in cherry tomato

3.1. Introduction

The rapid increase in population and the concerns about their health over the years have driven the demand for fresh vegetables and fruit, as they are the major source of essential minerals and vitamins for human wellbeing (Liplap et al., 2014; Mahajan et al., 2014). This has highlighted the role of the storage and transit conditions to maintain postharvest quality, including firmness, flavour, colour, shelf life and nutritional quality of the fresh produce (Fagundes et al., 2015; Liplap et al., 2014). Among all the postharvest environment components, such as relative humidity, ventilation and light, temperature has the greatest impact on the quality of fresh products (Brasil and Siddiqui, 2018). The management of an optimum temperature is the simplest and most important approach to delay deterioration of products (Brasil and Siddiqui, 2018).

The combination of low temperature and high relative humidity is one of the most used approaches to preserve postharvest quality of fresh products during storage and transit (Ayala-Zavala et al., 2004; Liplap et al., 2013). Low temperature is effective in reducing respiration and metabolic activity of products and inhibiting microbiological development (Brasil and Siddiqui, 2018). A lack of refrigeration is estimated to cause losses of between 15% and 40% of the total fresh produce production in the developed and developing countries, respectively (James and James, 2010; Liplap et al., 2014).

Although low temperature can extend the shelf life of vegetables and fruits, it can also cause chilling injuries, a term used to describe the physiological disorders, including surface pitting, uneven ripening, increased loss of aroma volatiles and water-soaked areas (Mangaraj et al.,

2019; Park et al., 2018b). It has been reported that tomato is susceptible to chilling injury at temperatures below 11ºC (Cheng and Shewfelt, 1988).

To avoid chilling injury and to extend shelf life, optimum temperatures are required. It has been reported that temperatures between 10 and 16°C were most suitable for the transit and storage of tomatoes, and temperatures between 18 and 21°C most suitable for ripening of tomato (Cantwell, 2000; Park et al., 2018b). However, these results relate to classic round tomatoes, and it isn't known if they are transferable to cherry tomatoes.

Three temperatures (5, 15, 20°C) were chosen from the range of chilling injury sensitive (below 11ºC), storage suitable (10 - 16°C) and ripening suitable (18 - 21°C) for classic round tomatoes, respectively, to examine the effect of temperature on nutritional quality of cherry tomatoes. This trial can not only provide standalone evidence on the effect of temperature but is also a precursor to later work where an optimum storage temperature was required.

3.2. Methods

3.2.1. Temperature treatments

See 2.1.2 for the details.

3.2.2. Appearance and weight loss

See 2.2 for the details.

3.2.3. Ascorbic acid

See 2.3.1 for the details.

3.2.4. Carotenoids (lycopene, β-carotene, and lutein)

See 2.3.2 for the details.

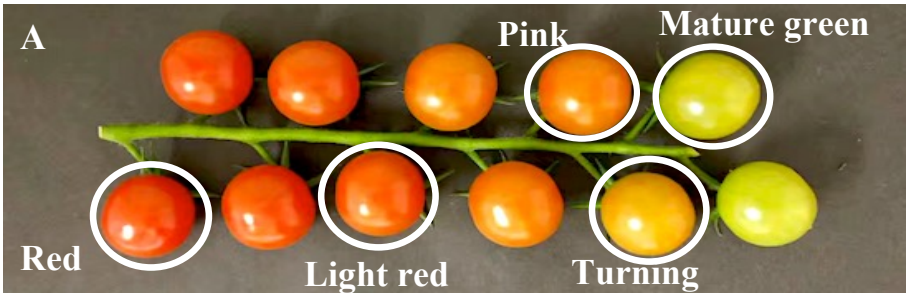
3.2.5. Total phenolics and flavonoids

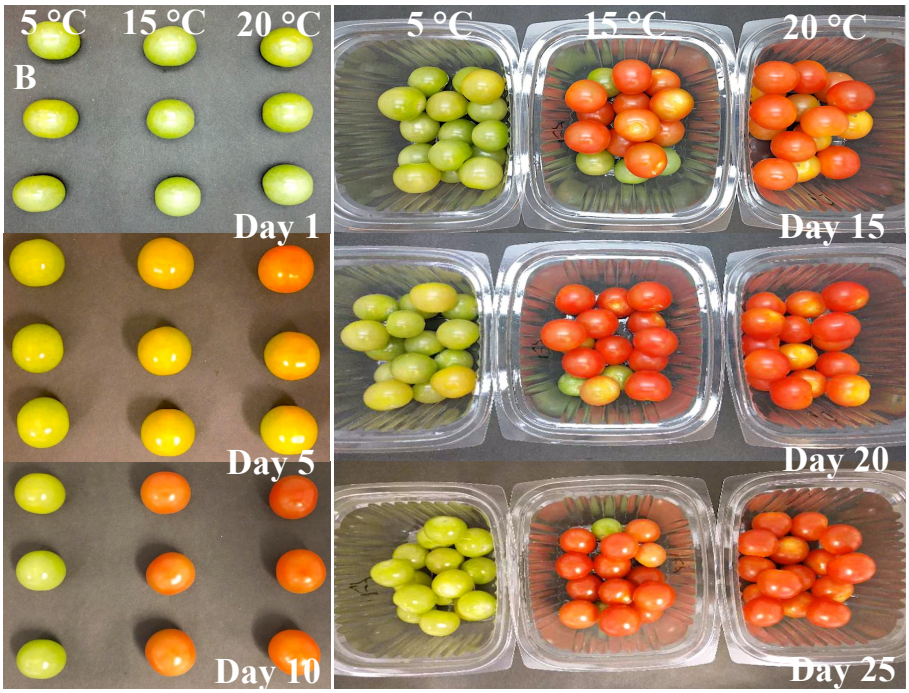
See 2.3.3 for the details

3.3. Results

3.3.1. Appearance

Fruit colour of cherry tomatoes was affected by storage temperature and time (Figure 3.1 B and C). Fruits stored at 15 and 20°C started turning colour from day 5, and most of them developed a fully red colour at the end of storage (day 25). Fruits stored at 5°C changed colour at day 10, but they failed to progress to the pink stage during storage, which is one of the symptoms of chilling injuries. When compared to 5 and 15°C, fruits stored at 20°C had the fastest ripening process, and no mature green tomatoes were found at day 15. These results show that fruits stored at 5°C failed to develop full colour and showed chilling injury symptoms, and that the temperature of 20°C is less effective in delaying ripening of cherry tomatoes when compared with 15°C.





C

Time	Temperature	Mature green	Turning	Pink	Light red	Red
Day 1	5 °C	15	0	0	0	0
	15 °C	15	0	0	0	0
	20 °C	15	0	0	0	0
Day 5	5 °C	15	0	0	0	0
	15 °C	9	6	0	0	0
	20 °C	9	5	1	0	0
Day 10	5 °C	14	1	0	0	0
	15 °C	4	0	5	6	0
	20 °C	0	9	4	1	1
Day 15	5 °C	10	5	0	0	0
	15 °C	2	2	3	5	3
	20 °C	0	2	5	5	3
Day 20	5 °C	10	5	0	0	0
	15 °C	1	1	2	0	11
	20 °C	0	0	2	4	9
Day 25	5 °C	10	5	0	0	0
	15 °C	1	0	1	2	11
	20 °C	0	0	0	2	13

Figure 3.1 **Effects of temperature on ripening of cherry tomato fruits. (A) Classification of ripening stage of cherry tomato fruits. (B) Appearance. (C) Ripening stages.**

3.3.2. Weight loss

Both storage time and temperature influenced weight loss in fruits (Figure 3.2). Weight loss increased steadily with time across all three temperatures. Fruits stored at 15°C had the lowest weight loss in all three treatments from day 10, with a value of 4.8% at the end of storage (day 25), while fruits stored at 5 and 20°C showed a similar value (7.6 and 7.4%, respectively) at day 25. There was an increase of 3.5% of weight loss in fruits stored at 20°C at day 10, leading to the highest weight loss in all treatments till day 20 ($P < 0.05$). The weight loss at 15°C was significantly lower than that at 5°C during storage, and that at 20°C from day 10 ($P < 0.05$).

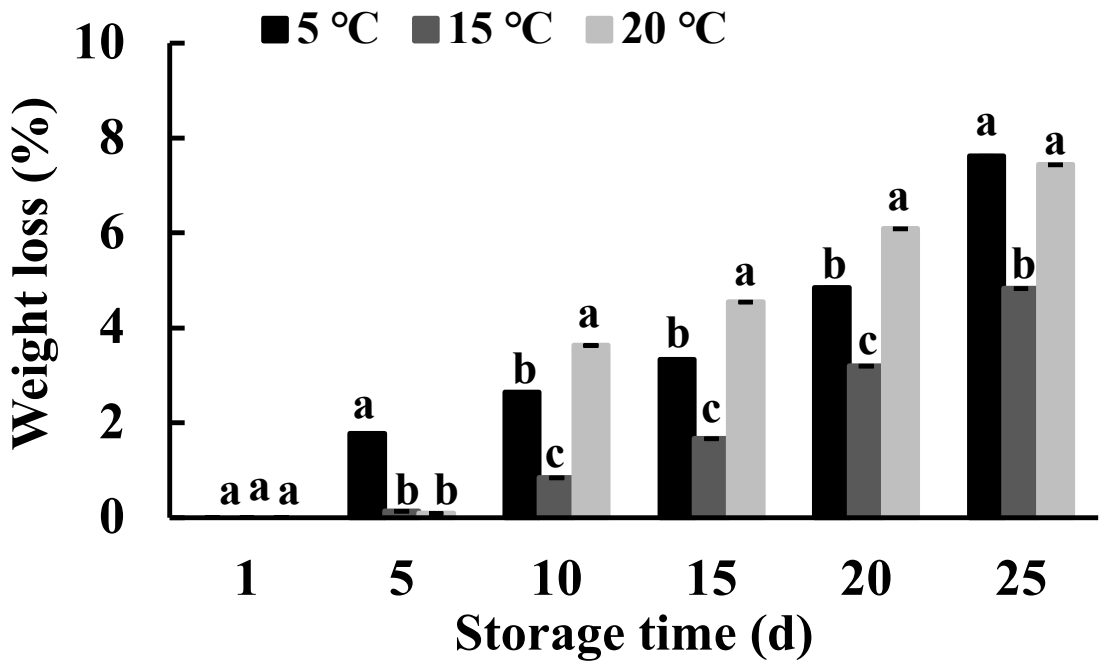


Figure 3.2 **Effects of temperature on weight loss of cherry tomato fruits during storage.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

3.3.3. Ascorbic acid

The content of ascorbic acid in fruits was affected by storage temperature and time (Table 3.1). Significant differences ($P < 0.05$) were found at three sampling days (day 10, 20 and 28) although ~~fluctuations~~ no obvious changes in ascorbic acid content were found over 28 days of storage (Figure 3.3). After 28 days of storage, ascorbic acid content increased from 1.6 to 3.2 mg/g at 20°C, followed by 15°C (2.6 mg/g) and 5°C (2.4 mg/g). The ascorbic acid content at 5°C remained consistent until day 25 but showed a sharp increase at day 28. This increase was also repeated at day 15 and 20°C, suggesting that a physiological or chemical shift took place between 25 and 28 days. Fruits stored at 20°C had the highest ascorbic acid content in all treatments for the first 10 days, and the content was significantly higher than that at 5°C at day 10 and 28 ($P < 0.05$).

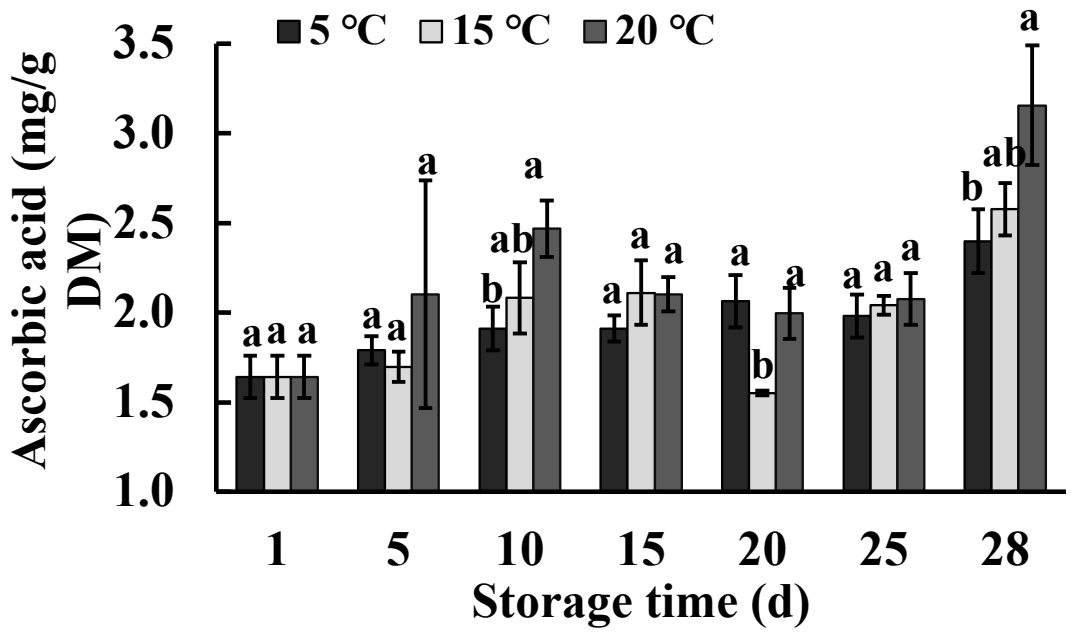


Figure 3.3 **Effects of temperature on ascorbic acid content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

3.3.4. Lycopene

Lycopene content was significantly affected by storage time and temperature ($P < 0.001$) (Table 3.1), with the content in fruits stored at 15 and 20°C accumulating greatly during storage (Figure 3.4). After 28 days of storage, lycopene content at 15 and 20°C increased from 1.3 to 29.6 and 24.9 mg/100 g, respectively. However, when stored at 5°C, the content remained constant (range between 0.4-3.8 mg/100 g) during 28 days of storage. Fruits stored at 5°C had the lowest lycopene level of all three treatments, and it was significantly lower than that at 15 and 20°C during the storage period ($P < 0.05$), suggesting that low temperature of 5°C inhibited production of lycopene. Lycopene content at 20 °C was highest in all treatments for the first 20 days, and then decreased, while at 15 °C, lycopene content accumulated steadily and reached the highest concentration of all treatments at the end of storage. This suggests that temperature of 15 °C is more suitable for long-term storage compared with 20 °C in terms of maintaining lycopene within the fruit..

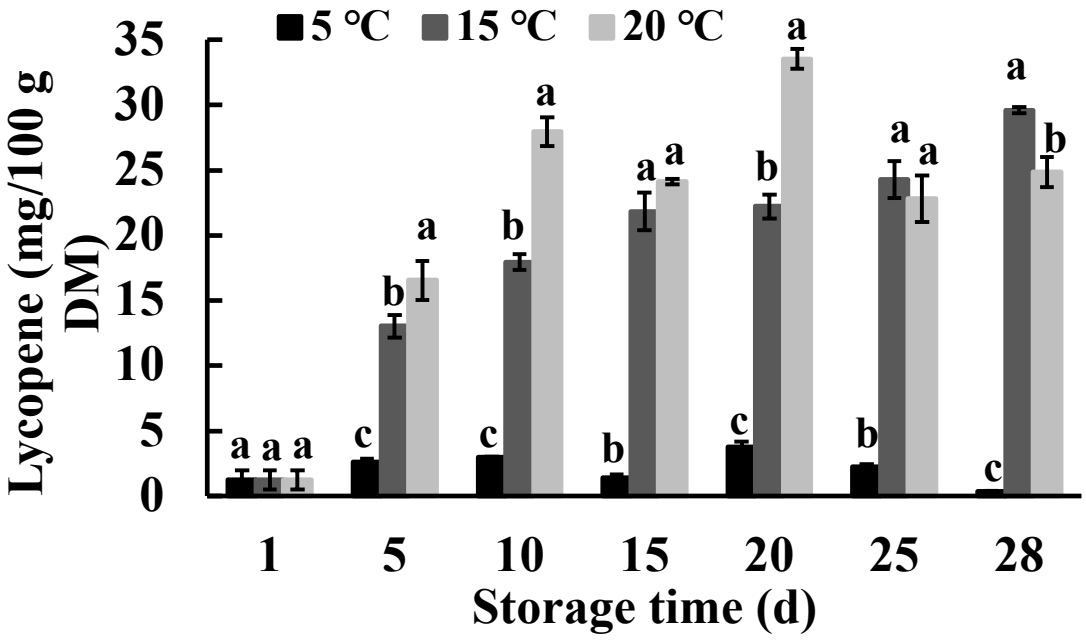


Figure 3.4 **Effects of temperature on lycopene content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

3.3.5. ß-carotene

ß-carotene content in fruits was significantly influenced by temperature and time ($P < 0.001$) (Table 3.1). Overall, contents remained fairly constant at 5°C, declining from 4.1 to 3.5 mg/100 g after 28 days of storage, and increasing at higher temperatures (Figure 3.5). The content of ß-carotene in fruits stored at 15 and 20°C increased to 13.8 and 9.3 mg/100 g, respectively, after 28 days of storage. ß-carotene content at 5°C was significantly lower than that at 15 and 20°C during the whole storage time ($P < 0.05$). These results suggest that low temperature of 5°C inhibited accumulation of ß-carotene. ß-carotene content at 15°C accumulated steadily and became the highest at day 25 and 28 in all treatments, and the content was significantly higher than that at 20°C at the end of storage ($P < 0.05$). Whereas at 20°C, the content decreased at the end of storage (day 28). This indicates that compared to 20°C, 15°C can delay the decrease of ß-carotene content and is more suitable for long-term storage of cherry tomatoes.

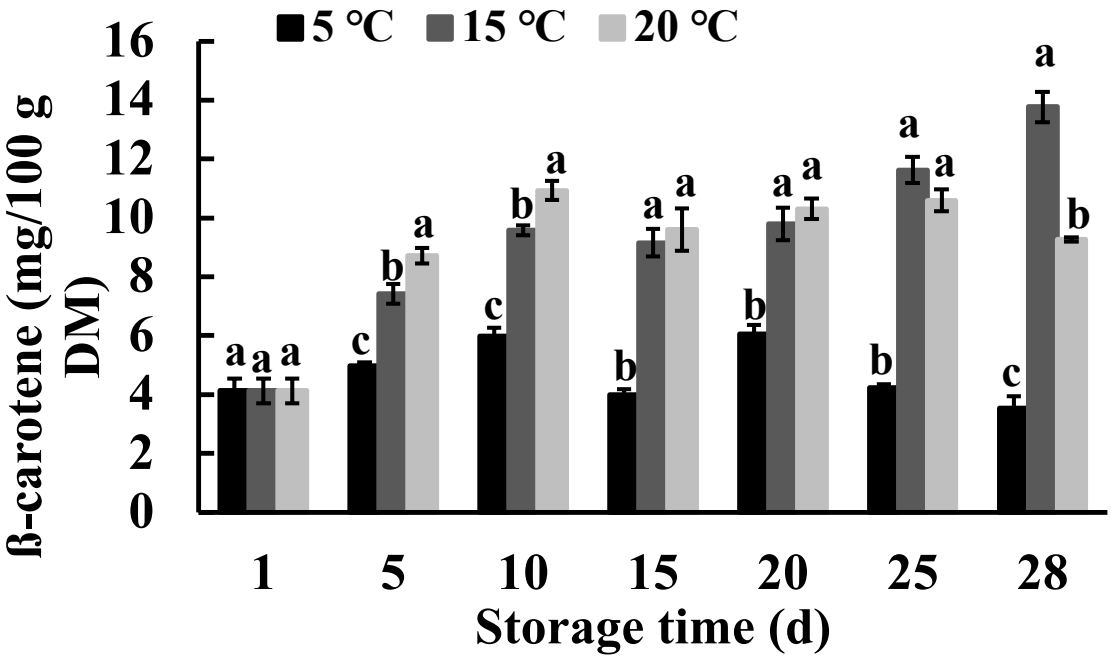


Figure 3.5 **Effects of temperature on ß-carotene content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

3.3.6. Lutein

Lutein content was not significantly affected by storage temperature (Table 3.1). During 28 days of storage, lutein content ranged between 5.7-8.1 mg/100 g (Figure 3.6). At 20°C, the content kept steady until day 25, followed by a decrease at day 28, with a final value of 5.7 mg/100 g, and the same lutein content was also found at 5°C at day 28 (5.7 mg/100 g). Whereas at 15°C, lutein content increased from 6.2 to 7.1 mg/100 g after 28 days of storage and became the highest in all treatments.

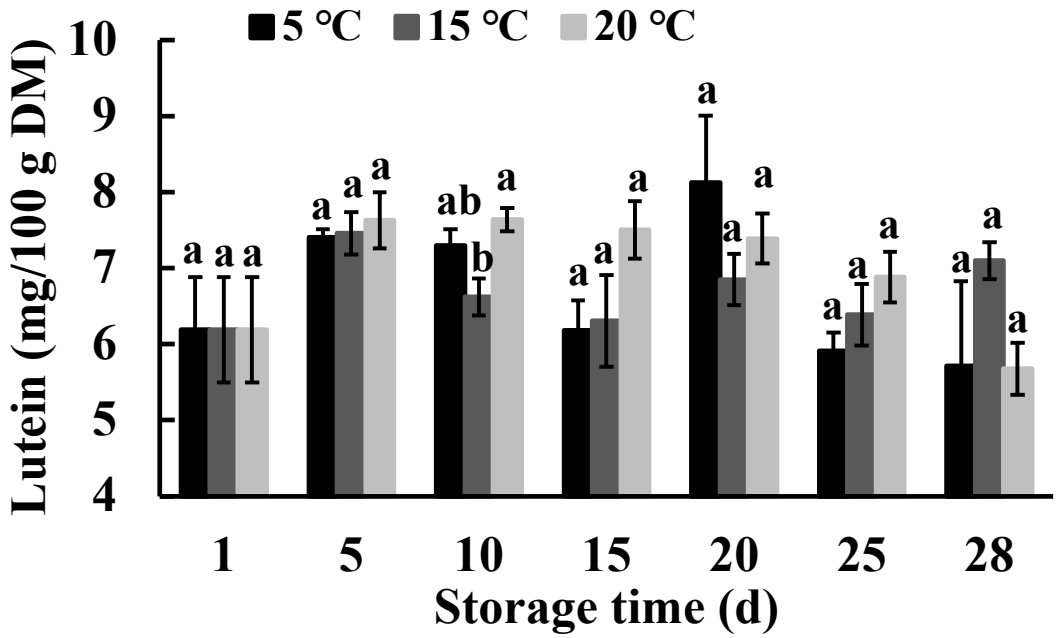


Figure 3.6 **Effects of temperature on lutein content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

3.3.7. Total phenolics

Temperature treatments and storage time both have a significant effect on the content of total phenolics ($P < 0.05$) (Table 3.1). The range of total phenolics content in fruits was between 31.2-46.2 mg/g during 28 days of storage (Figure 3.7). Total phenolics increased in all three temperatures after 28 days of storage, with the highest increase found at 20°C (7.4 mg/g), followed by 15°C (3.5 mg/g) and 5°C (2.5 mg/g). A significant difference was only found at day 5 where the content at 20°C was significantly higher than that 5°C.

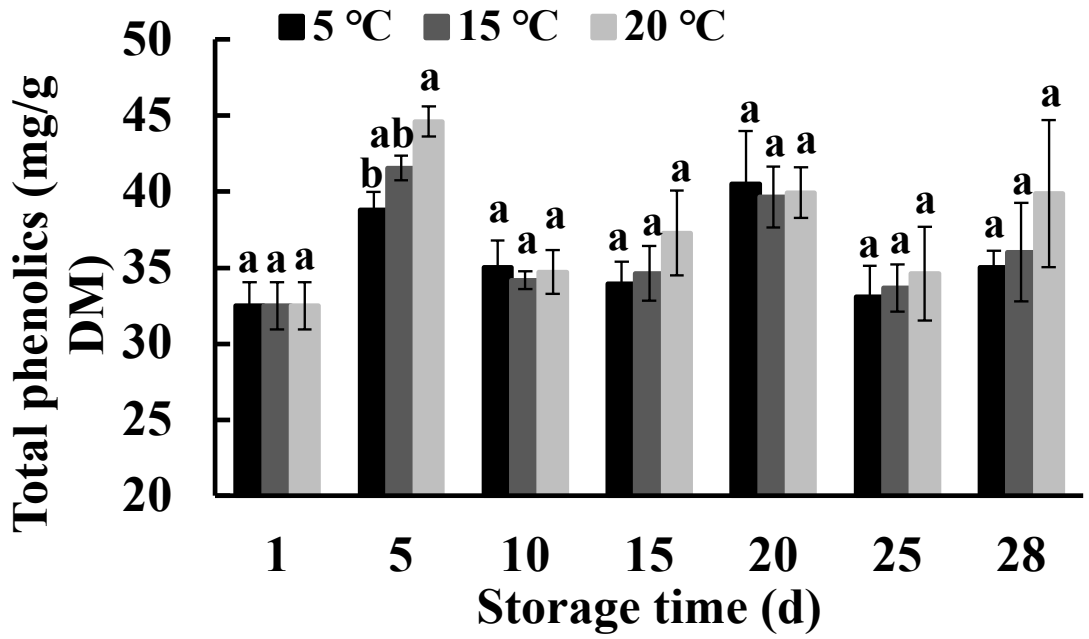


Figure 3.7 **Effects of temperature on total phenolics content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

3.3.8. Flavonoids

The content of flavonoids was not significantly affected by temperature treatments, but by storage time ($P < 0.05$) (Table 3.1). The range of flavonoids content in fruits was between 6.5-15.5 mg/g during 28 days of storage (Figure 3.8). The content decreased in all three

treatments after 28 days of storage, with the largest decrease found at 20°C (9.0 mg/g), followed by 5 (8.9 mg/g) and 15°C (6.7 mg/g). Although no significant differences were found between different temperatures, the content at 15°C was highest during 28 days of storage.

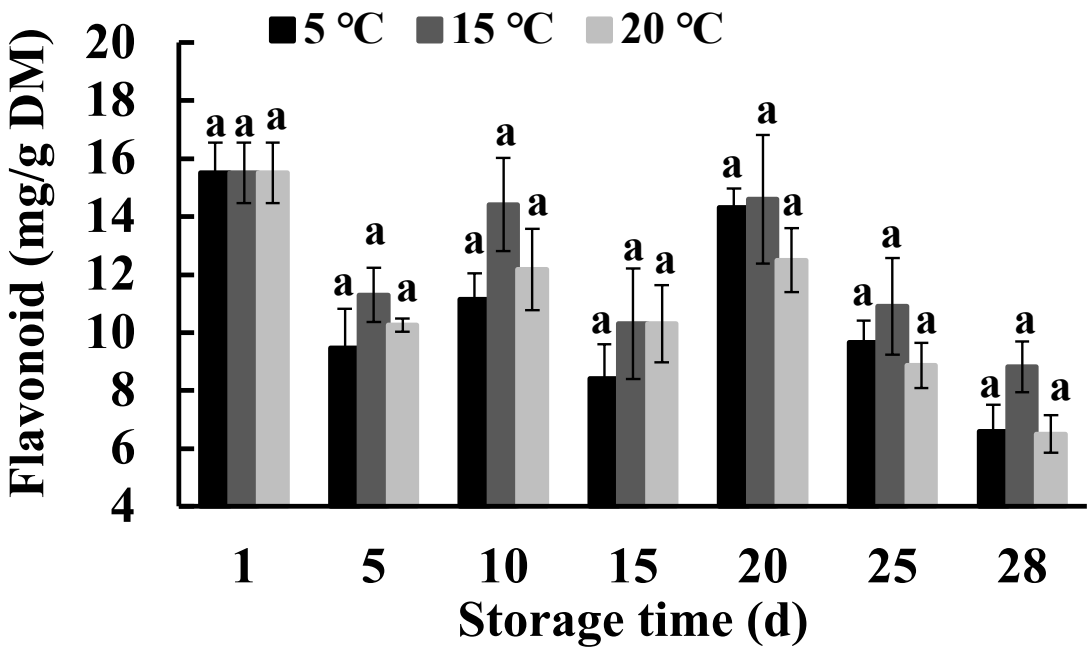


Figure 3.8 **Effects of temperature on flavonoids content in cherry tomato fruits during storage.** Values are the means ± SE for triplicate samples.

Table 3.1 Analysis of nutritional quality parameters of cherry tomato as affected by temperature and storage time.

	Temperature	Time	Temperature x Time
Ascorbic acid	***	***	ns
Lycopene	***	***	***
ß-carotene	***	***	***
Lutein	ns	***	**
Total phenolics	*	***	ns
Flavonoids	ns	***	ns

*, **, *** Significant differences at $P < 0.05$, 0.01, 0.001, respectively.

"ns", not significant.

3.4.Discussion

3.4.1. Appearance

Fruit colour turning, which is the result of the accumulation of lycopene and the degradation of chlorophyll, is one of the most important and obvious changes during cherry tomato ripening (Liu et al., 2011; Park et al., 2018a). External colour is the main basis of ripening stage classification of fruits, and it is also one of the most important quality criteria that contributes to the market value, as people's preference is for red tomatoes (Choi et al., 2015a; Panjai et al., 2017). In this study, fruits stored at 5°C failed to develop beyond the pink stage and showed chilling injury symptoms (such as poor colour development and premature softening) (Figure 3.1), which agrees with the finding of Gomez et al. (2009), who observed that cherry tomatoes stored at 6°C did not develop a red colour. Cantwell (2000) also reported that cherry tomatoes stored below 5°C for over a week failed in full colour development and showed chilling injury symptoms. This suggests that fruits stored under refrigerated condition (5°C) to lengthen storage-life is not suitable for mature green cherry tomato due to the failure in ripening and developing a red colour.

Storage temperature above 10°C ensures a full red colour development and avoids chilling injuries, but high temperatures (ripening temperatures above 25°C) have been shown to cause rapid softening, wilting, dehydration and a poor-colour of cherry tomatoes (Cantwell, 2000; Gomez et al., 2009). Cantwell (2000) reported that breaker stage tomato fruits stored at 15-20°C have longer shelf-life, more red colour and less decay than fruits stored at higher temperatures. But the comparison of the effect of 15 and 20°C on ripening and shelf-life of cherry tomato has not been reported. In this study, cherry tomatoes at 20°C had the fastest ripening process in all three treatments, and no mature green tomatoes were found at day 15

(Figure 3.1). From day 20, most fruits at 20°C were at red stage, and some of them started to soften at day 25. Whereas at 15°C, the ripening process was slower than that at 20°C. At day 25, most fruits were at red stage, and they were still very fresh (no shrink on fruit skin through visually observation). This suggests that compared to 20°C, 15°C is effective in delaying the ripening of cherry tomato and, as a consequence, extending the shelf-life.

3.4.2. Weight loss

Weight loss can lead to a shrivelled appearance of fruit skin and shortened shelf life, and subsequent economic value loss (Choi et al., 2015a; Javanmardi and Kubota, 2006; Kraśniewska et al., 2014). Fruits lost weight steadily during storage in all three temperatures (5, 15 and 20°C), with final losses at day 25 of 7.6%, 4.8% and 7.4%, respectively. At day 10, the weight loss at 5 and 20°C was 2.6 and 3.6%, respectively (Figure 3.2), which is consistent with Choi et al. (2015a), who reported weight loss of cherry tomatoes of around 2.8 and 4.9% at 4 and 20°C, respectively, after 9 days of storage. At day 15, the weight loss of cherry tomatoes stored at 5°C was 3.3% (Figure 3.2). Murmu and Mishra (2017) reported a lightly lower level of weight loss in tomatoes stored at 4°C after 14 days of storage, which was around 2.8% but their results are comparable with ours.

Fruits stored at 15°C had significantly lower weight loss than at 20°C during storage (Figure 3.2). Javanmardi and Kubota (2006) reported that weight loss at 12°C was significantly lower than that at room temperature (25-27°C). Mangaraj et al. (2019) found that tomatoes stored at 25°C had higher weight loss than at 10°C during 20 days of storage. The weight loss of fruits at 20°C was significantly higher than that at 5°C at most days of storage (Figure 3.2), which is consistent with Choi et al. (2015a), who reported that cherry tomatoes stored at 20°C had higher weight loss than at 4°C during storage. High respiration and transpiration rate of fruits might be the main cause of weight loss at 20°C (Guo et al., 2019; Murmu and Mishra, 2018).

Interestingly, weight loss of fruits stored at 15°C was significantly lower than that at 5°C during storage (Figure 3.2). The reason might be that fruits stored at 5°C developed physiological and biochemical disorders, such as reduce of membrane fluidity, loss of membrane integrity and ion leakage, leading to the loss of weight (Mangaraj et al., 2019; Park et al., 2018b).

3.4.3. Ascorbic acid

Ioannidi et al. (2009) found that ascorbic acid content of cherry tomatoes accumulated during fruit ripening. In this study, after 10 days of storage, ascorbic acid content in cherry tomato fruits (cv. Piccolo) increased from 1.6 to 2.5 mg/g at 20°C, followed by 15°C (2.1 mg/g) and 5°C (1.9 mg/g) (Figure 3.3). Toor and Savage (2006) reported that ascorbic acid in cherry tomatoes (cv. Tradiro) stored at 15 and 20°C increased slightly from an initial value of around 0.13 to 0.14 and 0.15 mg/g, respectively, after 10 days of storage, whereas at 7°C, it decreased slightly. But Gomez et al. (2009) found that ascorbic acid content of cherry tomatoes (cv. Micro-Tom) stored at 6 and 20°C showed modest decrease from an initial value of around 0.16 mg/g to 0.10 and 0.11 mg/g, respectively, after 15 days of storage. The differences in ascorbic acid content and their changes during storage might be related to the cultivar of tomato.

Fruits stored at 20°C had the highest ascorbic acid content across all treatments for the first 10 days, and the content was significantly higher than that at 5°C at day 10 and 28 ($P < 0.05$) (Figure 3.3). Toor and Savage (2006) reported similar result that ascorbic acid content in tomatoes stored at 25°C was higher than that at 15 and 7 °C. Gomez et al. (2009) also found that although no significant differences were shown, ascorbic acid content in tomatoes at 20°C was higher than that at 4°C after 15 days of storage. The content of ascorbic acid at

15°C was higher than that at 5°C at most days analysed during storage (Figure 3.3). We are not aware of any previous publication highlighted this finding.

3.4.4. Lycopene

Lycopene is the most abundant carotenoid in red cherry tomatoes and causes the increase in redness during fruit ripening (Liu et al., 2011; Toor and Savage, 2006). After 15 days of storage, lycopene content in cherry tomatoes (cv. Piccolo) stored at 15 and 20°C increased from 1.3 to 21.8 and 24.1 mg/100 g, respectively (Figure 3.4). Aljouni et al. (2001) reported a lower level in tomatoes (cv. Pyramid) stored at 22°C, which increased from 3.6 to 9.0 mg/100 g after 14 days of storage. Toor and Savage (2006) also reported that lycopene content of cherry tomatoes (cv. Tradiro) stored at 15 and 25°C increased from around 3 to 6.8 and 8.2 mg/100 g, respectively, after 10 days of storage. The differences in lycopene content during storage might be related to the cultivar of tomato. In this study, lycopene concentration at 5°C remained fairly constant during storage (Figure 3.4), which was consistent with the finding of Javanmardi and Kubota (2006), who reported that no significant changes of lycopene content were found in tomatoes stored at low temperature (5°C). Aljouni et al. (2001) reported similar results in tomatoes stored at 4°C.

The accumulation of lycopene can be significantly affected by storage temperature (Park et al., 2018a). In this study, cherry tomatoes stored at 5°C had significantly the lowest lycopene content in all three treatments during 28 days' storage (Figure 3.4), which corresponds well with the study of Javanmardi and Kubota (2006), who reported that low temperature of 5°C inhibited accumulation of lycopene, and the content was significantly lower than that at 12°C. Toor and Savage (2006) reported a similar result that cherry tomatoes stored at 15 and 25°C had higher lycopene content than those stored at 7°C. Choi et al. (2015a) found that lycopene content in cherry tomatoes stored at 20°C was higher than that at 4°C. It has been

reported that temperatures below 12°C strongly inhibit the synthesis of lycopene, and temperatures above 32°C can block it completely (Dumas et al., 2003; Fagundes et al., 2015).

In this study, lycopene concentration at 20°C decreased after day 20, whereas at 15°C, lycopene content accumulated steadily and reached the highest concentration of all treatments at the end of storage (Figure 3.4), indicating that temperature of 15°C can delay the decrease of lycopene content, and is more suitable for long-term storage compared with 20°C in terms of lycopene content.

3.4.5. β-carotene

β-carotene, constituting 5%-10% of the total carotenoids, is responsible for the orange colour of cherry tomatoes (Schofield and Paliyath, 2005). The content of ß-carotene in fruits stored at 15 and 20°C increased from 4.1 to 9.8 and 10.3 mg/100 g, respectively, after 20 days of storage, whereas at 5°C, the content remained unchanged (Figure 3.5). Panjai et al. (2017) found similar result that ß-carotene in tomato stored at 20°C increased from around 4.7 to 7.8 mg/100 g after 20 days of storage. Park et al. (2018a) studied ß-carotene content change in black tomatoes stored at 20°C, and found that the content increased from 4.4 to 12.8 mg/100 g after 13 days of storage, and then decreased to 2.6 mg/100 g at day 20. The quick decrease might be a special characteristic of black tomato.

In this study, ß-carotene at 5°C was significantly lower than that at 15 and 20°C during the whole storage time, suggesting that low temperature of 5°C inhibited the production of ß-carotene. Cherry tomatoes stored at 15°C had the highest ß-carotene level in all three treatments after 28 days storage (Figure 3.5). Park et al. (2018a) found similar results that in all three temperatures (8, 12, 20°C), 12°C was the optimal storage temperature for ß-carotene synthesis in black tomatoes.

In this study, the changing pattern of β-carotene was similar as that of lycopene (Figure 3.5). At 20°C, β-carotene content decreased after day 20, whereas at 15°C, it accumulated steadily and reached the highest concentration of all treatments at the end of storage. This is because lycopene can be converted to ß-carotene by the action of enzyme LCY-b.

3.4.6. Lutein

In this study, lutein content decreased at both 5 and 20°C after 28 days of storage (Figure 3.6). Liu et al. (2018) reported similar result that lutein content in tomatoes stored at 25°C decreased during 11 days of storage. Lutein content was not significantly affected by storage temperature in this study (Figure 3.6), which is in accordance with the finding of González-Casado et al. (2018), who reported that temperatures of 4, 12 and 20°C had no significant effect on lutein content in tomatoes during storage.

At the end of storage (day 28), cherry tomatoes stored at 15°C had the highest content of lycopene, β-carotene and lutein content in all three temperatures (Figure 3.6), suggesting that a temperature of 15°C is more suitable for long-term storage in comparison to 5 and 20°C in terms of carotenoids content. We are not aware of any previous publication highlighted this finding.

3.4.7. Total phenolics

In plants, phenolic compounds are important secondary metabolites, and their antioxidative activities help reduce the incidence of cardiovascular diseases in humans (Liu et al., 2011). In cherry tomatoes (cv. Piccolo), the range of total phenolics content was between 31.2-46.2 mg/g during 28 days of storage (Figure 3.7). Luthria et al. (2006) reported a similar amount of total phenolics content at mature tomatoes, which was 40 mg/g in Oregon Spring cultivar

and 33 mg/g in Red Sun cultivar at harvest. However, Panjai et al. (2017) reported a lower content of total phenolics in tomato (cv. Cappricia) stored at 20°C, which was between 3.5 to 5.7 mg/g during 20 days of storage. This means that total phenolics content is cultivar dependent.

Total phenolics in cherry tomatoes (cv. Piccolo) increased at all three temperatures after 28 days of storage, with the highest increase found at 20°C, followed by 15°C and 5°C, and the content at 20°C was significantly higher than that at 5°C at day 5 (Figure 3.7). Ayala-Zavala et al. (2004) found a similar result that total phenolics content in strawberries stored at 5 and 10°C increased during 13 days of storage, and the content at 10°C was higher than that at 5°C. Toor and Savage (2006) also report that phenolic content in cherry tomatoes (cv. Tradiro) stored at 7, 15 and 25 °C increased for the first 8 days, and then declined toward the end of the storage, and the content at 25 and 15 °C was higher than that at 7°C after 10 days of storage.

3.4.8. Flavonoids

Flavonoids are the largest group of phenolic compounds in tomato fruits and contribute to fruit colour, fragrance, and aroma (Raiola et al., 2014). In cherry tomatoes (cv. Piccolo), the range of flavonoids content was between 6.5-15.5 mg/g during 28 days of storage (Figure 3.7). Panjai et al. (2017) reported a similar value of flavonoids content in tomato (cv. Cappricia) stored at 20°C, which was between 11.2 to 14.9 mg/g during 20 days of storage. But Liu et al. (2011) found a lower flavonoids content in tomato (cv. Zhenfen) stored at 15°C, which ranged from 0.07 to 0.12 mg/g during 37 days of storage. Toor and Savage (2006) also report that flavonoids content in cherry tomatoes (cv. Tradiro) stored at 7, 15 and 25 °C was between 0.05 to 0.08 mg/g during 10 days of storage. This means that same as total phenolics, flavonoids content is also cultivar dependent.

The flavonoid content decreased in all three treatments after 28 days of storage, with the largest decrease found at 20°C, followed by 5 and 15°C, and the content at 15°C was highest during 28 days of storage, although there were no significant differences between the temperature treatments (Figure 3.8). Toor and Savage (2006) found a similar result that total soluble flavonoids in tomatoes stored at 7, 15 and 25°C was not significantly influenced by temperatures.

3.5. Conclusion

Storage time significantly affected nutritional quality of cherry tomatoes ($P < 0.05$), while storage temperature showed significant effects on the content of ascorbic acid, lycopene, ß-carotene and total phenolics ($P < 0.05$). Storage temperature of 20 °C led to high weight loss, fast ripening, and rapid increase of nutritional quality, including lycopene, ß-carotene and lutein, followed by a later decrease, whereas storage temperature of 15 °C inhibited weight loss and the decrease of lycopene, ß-carotene, lutein and flavonoids in cherry tomatoes observed after prolonged storage at 20 °C. Temperature of 5 °C inhibited accumulation of nutritional quality and caused chilling injuries (failure in colour development). In conclusion, in comparison to the other two temperatures, 15 °C prolonged shelf life and preserved nutritional quality of cherry tomatoes, suggesting it is the best one in all three storage temperatures to maintain fruit quality during long-term storage. This temperature will be used for later experimentation.

4. Influence of postharvest red/far-red, blue light and their irradiation time on nutritional quality of cherry tomato

4.1. Introduction

As a climacteric fruit, cherry tomato continues to ripen after being harvested, undergoing physiochemical changes such as softening, colour evolution, and aroma development, and subsequently loss of quality (Domínguez et al., 2016; Kaewklin et al., 2018). Postharvest treatment is therefore required to preserve fruit quality during storage and transportation and to prolong shelf life. Light irradiation has been proposed as an effective postharvest treatment (Hasan et al., 2017).

Light is a crucial factor that controls physiological and biochemical responses of plants (Hasan et al., 2017; Ma et al., 2014). It has been demonstrated that a controlled amount of light could increase the content of nutrients and bioactive compounds production to improve postharvest quality of shelf-life of crops (Braidot et al., 2014; Costa et al., 2013; Glowacz et al., 2015; Hasan et al., 2017). Light-emitting diodes (LEDs) with narrow wavelengths are a promising tool in food preservation (Hasan et al., 2017; Morrow, 2008).

The application of single-spectral red or blue light to post-harvest climacteric fruits can delay senescence by reducing the production of ethylene and ascorbates (Hasan et al., 2017; Ma et al., 2014). It has been demonstrated that single-spectral blue light could control stomatal opening, and some pigments synthesis in postharvest fruits (Gong et al., 2015; Ma et al., 2014; Xu et al., 2012). Blue light could also induce the accumulation of vitamin C, chlorophylls, soluble sugar, and soluble protein in Chinese cabbage (Li et al., 2012). Although blue light has been reported to maintain higher level of firmness and free amino

acids compared to dark condition in postharvest tomatoes (Dhakal and Baek, 2014a), little information is available about the effect on other nutritional components in cherry tomatoes.

Red and far-red light can regulate plant development and anthocyanin biosynthesis in postharvest vegetables and fruits (Alba et al., 2000a; Ma et al., 2014). Red light can also delay the yellowing and reduce ethylene production in postharvest broccoli and induce the content of β-cryptoxanthin in citrus fruit (Ma et al., 2012; Ma et al., 2014). Red light has been reported to induce the content of carotenoid in tomatoes, whereas red/far-red light has a reverse effect (Alba et al., 2000a; Liu et al., 2009). However, there is little information about the effect of high ratio of red/far-red light on nutritional quality in cherry tomatoes.

The aim of this chapter was to investigate the effect of postharvest red/far-red or blue light irradiation, and the irradiation time (short-, middle- and long-duration red/far-red or blue light irradiation) on cherry tomatoes' nutritional quality, including the content of ascorbic acid, lycopene, β-carotene, lutein, total phenolics and flavonoids.

4.2. Methods

4.2.1. Light treatments

See 2.1.3 for the details.

4.2.2. Appearance and weight loss

See 2.2 for the details.

4.2.3. Ascorbic acid

See 2.3.1 for the details.

4.2.4. Carotenoids (lycopene, β-carotene, and lutein)

See 2.3.2 for the details.

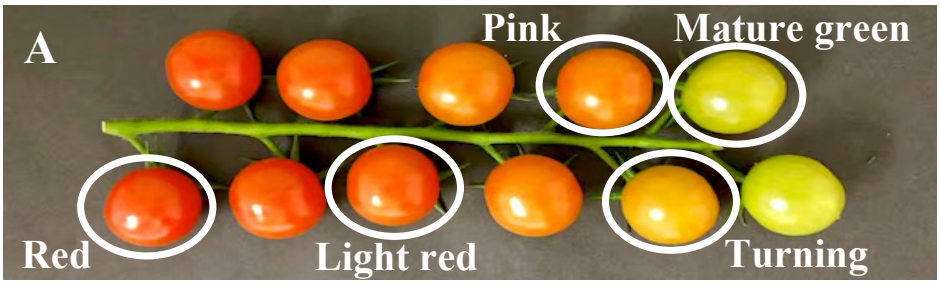
4.2.5. Total phenolics and flavonoids

See 2.3.3 for the details

4.3. Results

4.3.1. Appearance

Fruit colour of cherry tomatoes was affected by treatments and time (Figure 4.1 B and C). Fruits treated with long-duration red/far-red light reached the pink stage at day 5, whereas in long-duration blue light treatment and dark control, fruits were still at mature green and turning stage. Compared with the control, fruits treated with red/far-red light showed deeper red colour from day 5 to 25, with most fruits turning red from day 15. Fruits treated with blue light had a similar ripening timecourse as the control, with a similar number of red fruits from day 5 to 20. These results show that red/far-red light promoted the colour change and ripening of fruits, while blue light had little effect on it.



C

Time	Treatments	Mature green	Turning	Pink	Light Red	Red
Day 0	Dark	15	0	0	0	0
	Blue	15	0	0	0	0
	R/FR	15	0	0	0	0
Day 5	Dark	9	6	0	0	0
	Blue	8	7	0	0	0
	R/FR	5	8	2	0	0
Day 10	Dark	7	2	1	4	1
	Blue	3	1	5	4	2
	R/FR	2	1	2	6	4
Day 15	Dark	2	5	2	1	5
	Blue	3	2	3	3	4
	R/FR	1	2	2	3	7
Day 20	Dark	0	6	1	2	6
	Blue	0	3	1	4	7
	R/FR	0	1	2	2	10
Day 25	Dark	0	0	2	5	8
	Blue	0	0	3	1	11
	R/FR	0	0	1	2	12

Figure 4.1 **Effects of long-duration red/far-red and blue light on ripening of cherry tomato fruits stored at 15°C. (A) Classification of cherry tomato fruit ripening stages. (B) Appearance. (C) Ripening stages.**

4.3.2. Weight loss

Fruits in all treatments lost weight throughout the storage period (Figure 4.2). After 25 days of storage, losses were 3.0%, 4.3% and 3.6% for the long-duration red/far-red light and blue light treatment, and dark control, respectively. Fruits treated with red/far-red light had the lowest weight loss during storage, and it was significantly lower than that in control at day 15 ($P < 0.05$). Blue light treatment caused the highest weight loss during storage, and the loss was significantly higher than that in control at day 15 and 20 ($P < 0.05$). These results show that red/far-red light inhibited weight loss, whereas blue light promoted it.

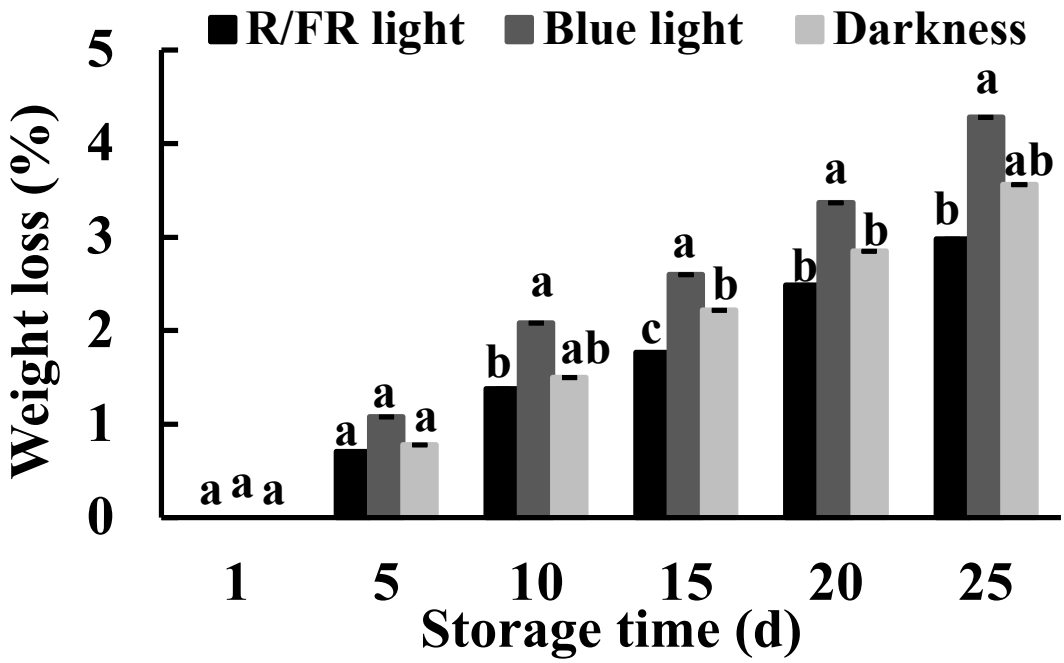


Figure 4.2 **Effects of long-duration red/far-red and blue light on weight loss of cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.3. Ascorbic acid

The content of ascorbic acid in fruits was affected by storage temperature and time (Table 4.1). Ascorbic acid content, despite some divergence at day 10, remained fairly constant between the treatments (1.5-2.1 mg/g) until day 25 when it increased in the light treatments to over 3 mg/g (Figure 4.3). This increase was short-lived as concentrations returned to their baseline at day 33, with the highest content in long-duration red/far-red light treatment (2.2 mg/g), followed by blue light treatment (1.9 mg/g) and control (1.9 mg/g). The content of ascorbic acid in red/far-red light treatment was higher than that in control at most sampling days, but significant difference was only found at day 10. There were no significant differences between blue light and the darkness control during storage. These results show that red/far-red light increased ascorbic acid content, whereas blue light had little effect on it.

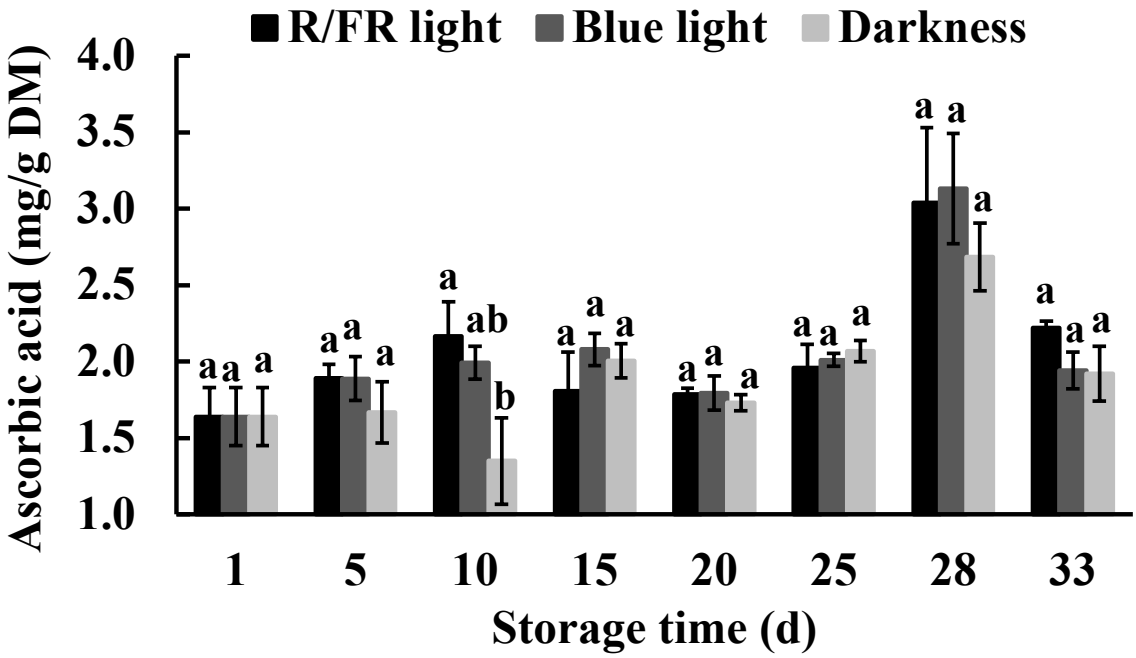


Figure 4.3 **Effects of long-duration red/far-red and blue light irradiation on ascorbic acid content in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

The duration of the red/far-red light treatment had no significant influence on ascorbic acid content during storage (Table 4.1). Ascorbic acid content, despite some divergence at day 28, remained fairly constant in all three treatments (Figure 4.4). At day 28, the highest ascorbic acid content was found in R/FR-M treatment (3.1 mg/g), followed by R/FR-L (3.0 mg/g) and R/FR-S (2.6 mg/g) treatment. The content in R/FR-S treatment was also the lowest in all three treatments at day 33 (2.1 mg/g), while in R/FR-M and R/FR-L treatment, it was 2.4 and 2.2 mg/g, respectively.

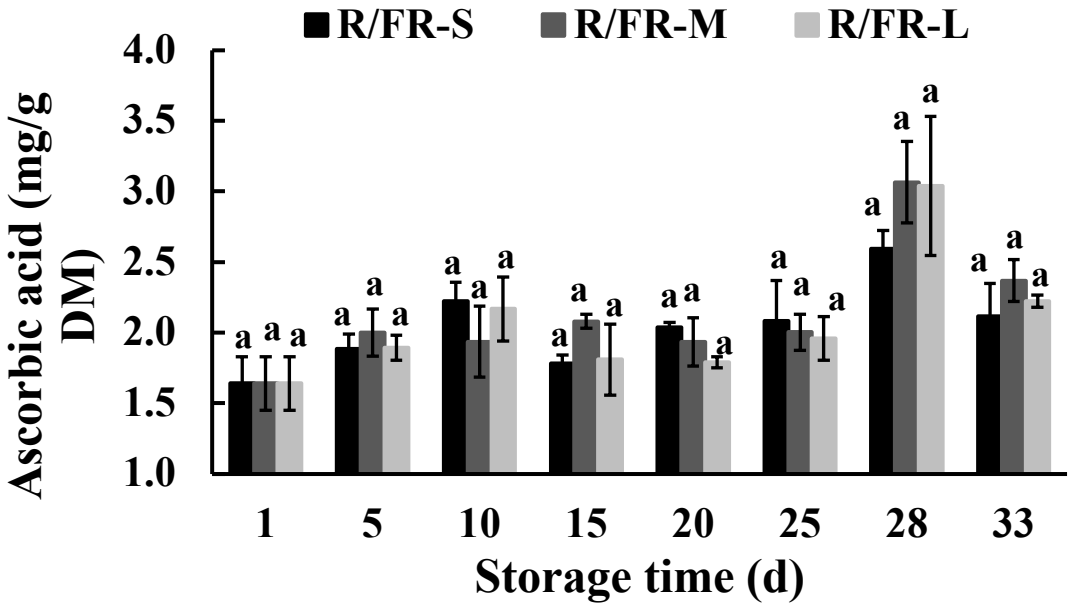


Figure 4.4 **Effects of red/far-red light irradiation time on ascorbic acid content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

The duration of the blue light treatment had no significant influence on ascorbic acid content during storage (Table 4.1). Ascorbic acid content, despite a divergence at day 28, remained fairly constant in all three treatments (Figure 4.4). At day 28, the highest ascorbic acid content was found in B-L treatment (3.1 mg/g), followed by B-M (2.9 mg/g) and B-S (2.9

mg/g) treatment. While at day 20 and 25, the B-M had the highest ascorbic acid content. The content in B-M treatment was also the highest in all three treatments at day 33 (2.1 mg/g), followed by B-S (2.1 mg/g) and B-L treatment (1.9 mg/g).

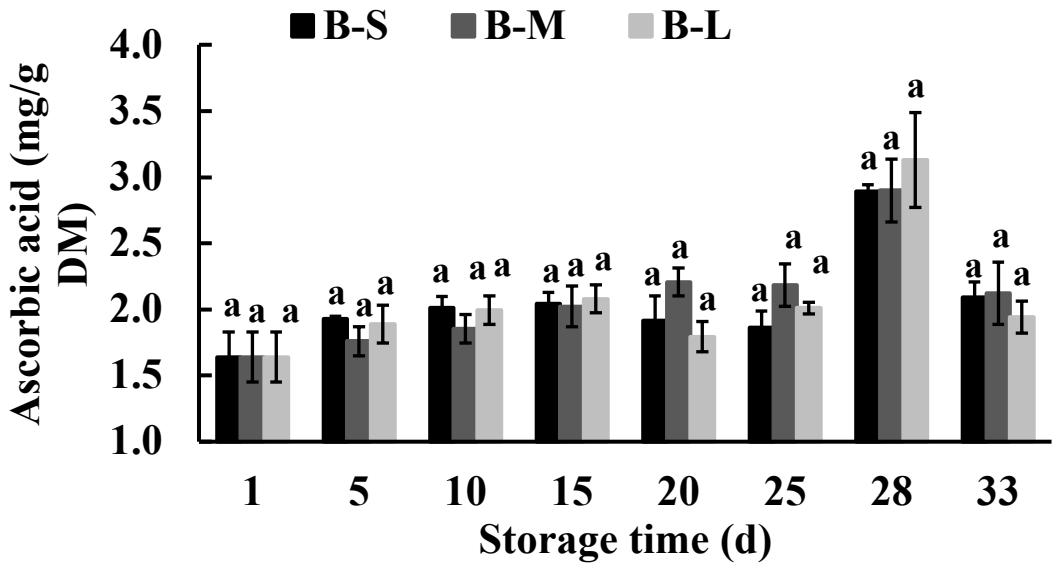


Figure 4.5 **Effects of blue light irradiation time on ascorbic acid content in cherry tomato fruits during storage at 15°C.** B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.4. Lycopene

Lycopene content was significantly influenced by storage time and light treatments ($P < 0.001$) (Table 4.1). Lycopene content increased steadily over the storage period (Figure 4.6). After 33 days of storage, it had risen from 1.2 to 36.9, 34.9 and 33.7 mg/100 g in the red/far-red light treatment, blue light treatment and control, respectively. The highest lycopene content was found in the red/far-red light treatment at day 28, with the value of 40.6 mg/100 g. The content of lycopene in red/far-red light treatment was higher than that in control from day 10, and significant differences were found at day 25 and 28 ($P < 0.05$). While in blue

light treatment, the content showed no significant difference with that in control from day 10. These results show that red/far-red light increased lycopene content during storage compared to darkness at 25 and 28 days after storage, whereas blue light had no significant effect on it.

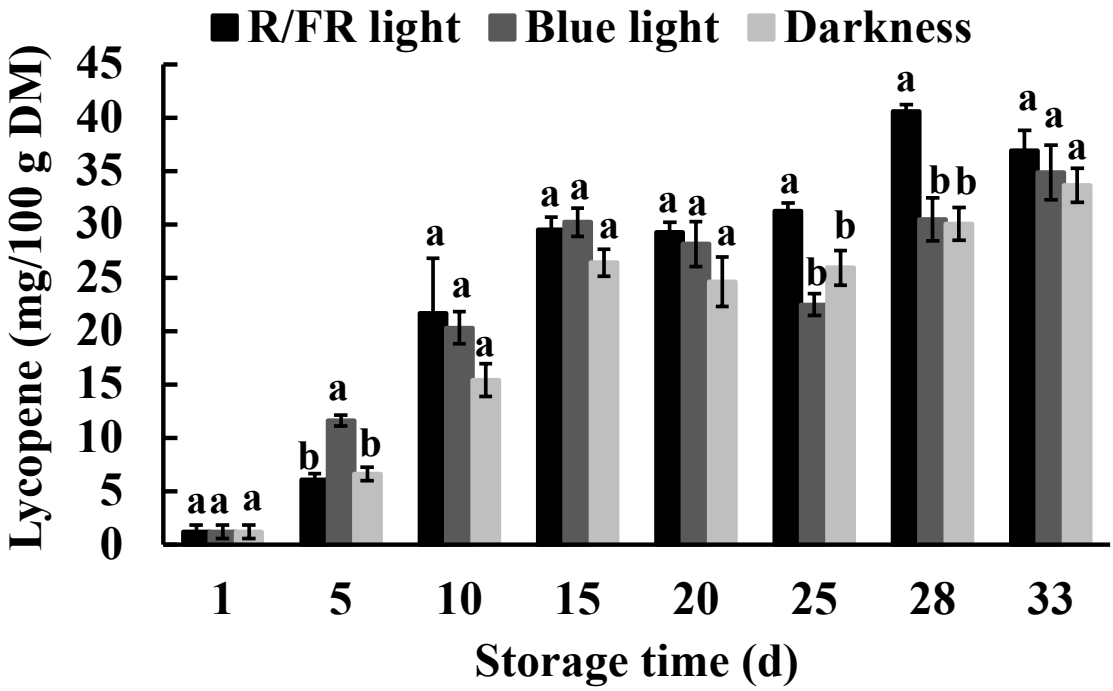


Figure 4.6 **Effects of long-duration red/far-red and blue light irradiation on lycopene content in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Lycopene content was significantly affected by red/far-red light irradiation time ($P < 0.05$) (Table 4.1). Lycopene content increased steadily over the storage period in all three treatments (Figure 4.7). At day 33, the highest lycopene content was found in R/FR-M treatment (40.6 mg/100 g), followed by R/FR-L (36.9 mg/100 g) and R/FR-S treatment (35.7 mg/100 g). The content in R/FR-L treatment was the highest in all treatments at day 15 and 28, and it was significantly higher than that in R/FR-S treatment ($P < 0.05$). While in R/FR-M treatment, the content was higher than that in R/FR-S treatment at sampling days during

storage although no significant difference was observed at most days. The content in R/FR-L treatment had no significant difference with that in R/FR-M treatment during storage.

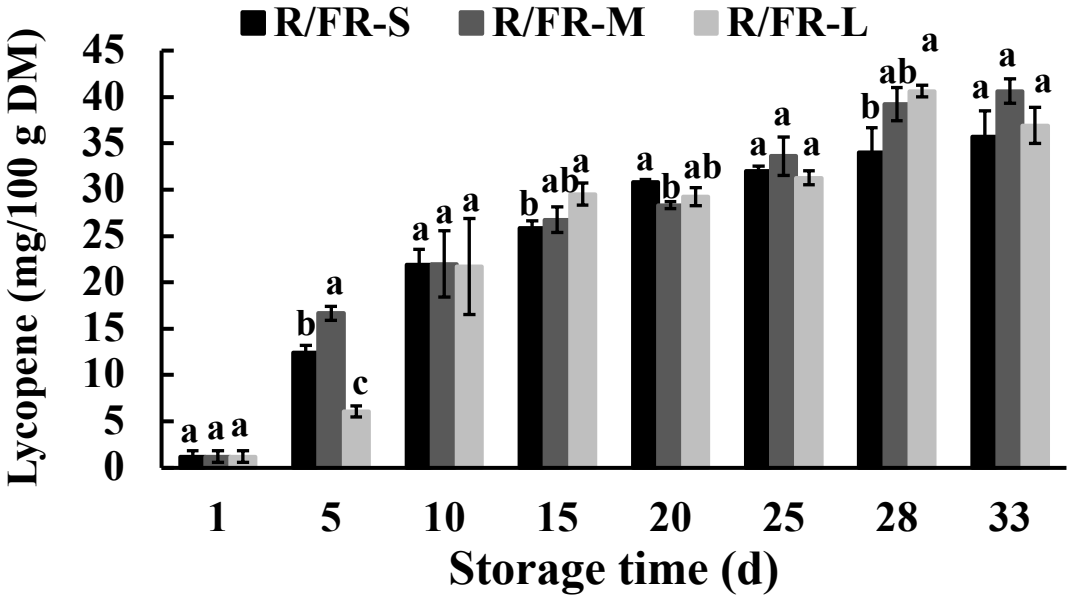


Figure 4.7 **Effects of red/far-red light irradiation time on lycopene content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Lycopene content was significantly affected by blue light irradiation time ($P < 0.05$) (Table 4.1). Lycopene content increased steadily over the entire storage period in all three treatments (Figure 4.8). At day 33, the highest lycopene content was found in B-M treatment (36.1 mg/100 g), followed by B-L (34.9 mg/100 g) and B-S treatment (34.7 mg/100 g). Lycopene content in B-L treatment was significantly higher than that in B-S treatment at day 10 and 15 ($P < 0.05$), but no significant difference was found afterwards. In the R/FR-M treatment, the content was higher than that in R/FR-S treatment, and a significant difference was observed at day 10. There was no significant difference in lycopene content between B-L and B-M treatment.

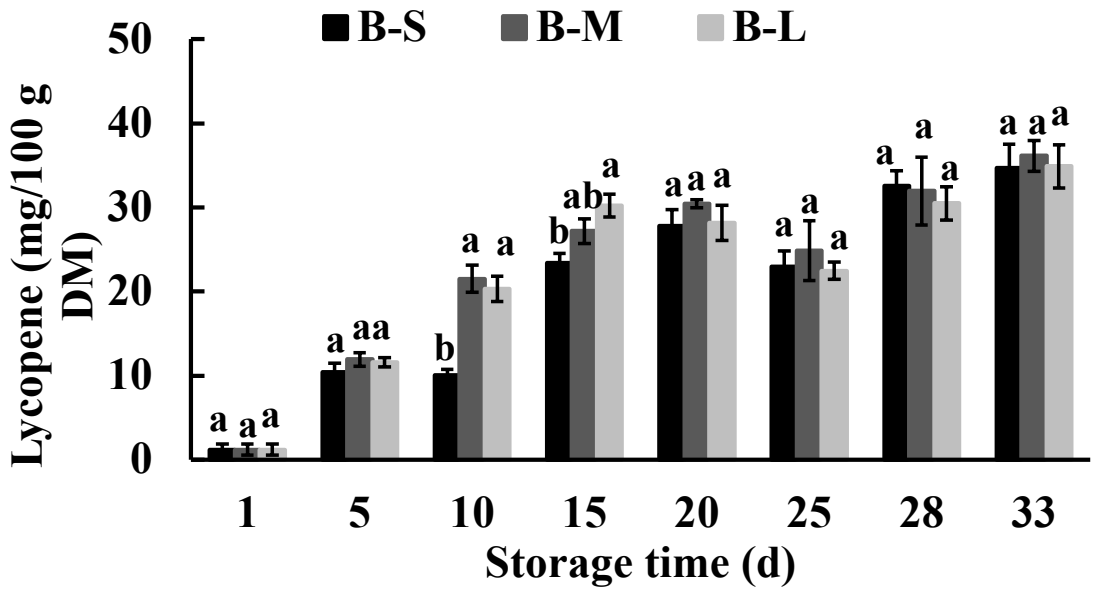


Figure 4.8 **Effects of blue light irradiation time on lycopene content in cherry tomato fruits during storage at 15°C.** B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.5. ß-carotene

ß-carotene content was significantly affected by storage time and light treatments ($P < 0.001$) (Table 4.1). The content of ß-carotene accumulated steadily over the entire storage time (Figure 4.9). After 33 days' storage, it had risen from 4.5 to 24.2, 16.7 and 14.4 mg/100 g in the red/far-red light treatment, blue light treatment and control, respectively. Fruits treated with red/far-red light had the highest β-carotene content from day 10 to 33, and the content was significantly higher than that in control from day 15 to 33 ($P < 0.05$). In blue light treatment, ß-carotene content was significantly higher than that in control at day 5, 15 and 20 ($P < 0.05$), but no significant difference was shown afterwards. These results show that red/far-red light increased ß-carotene content during storage compared to darkness, whereas blue light had little effect on it after day 20.

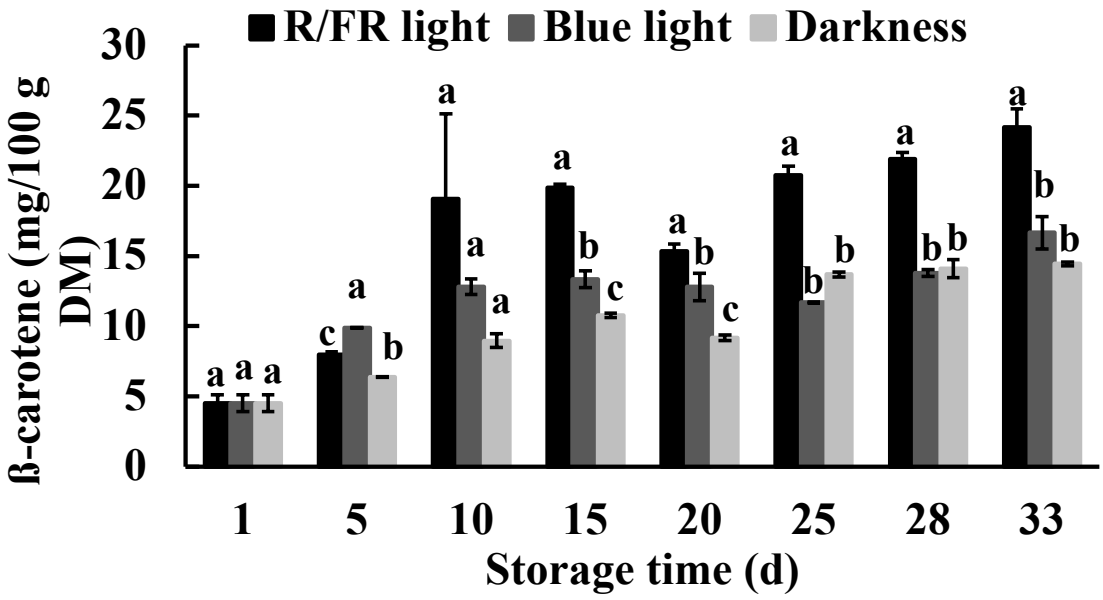


Figure 4.9 **Effects of long-duration red/far-red and blue light irradiation on β-carotene content in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

β-carotene content was significantly affected by red/far-red light irradiation time ($P < 0.05$) (Table 4.1). β-carotene content increased over the entire storage period in all three treatments (Figure 4.10). After 33 days of storage, it had risen to 24.2, 23.5 and 21.5 mg/100 g in the R/FR-L, R/FR-M treatment and R/FR-S treatment, respectively. Fruits treated with R/FR-L had the highest β-carotene content which was significantly higher than that in R/FR-S treatment at day 15, 20 and 28 ($P < 0.05$). The content in R/FR-M treatment was higher than that in R/FR-S treatment at most sampling points and a significant difference was observed at day 10. Fruits treated with R/FR-L had similar β-carotene content with that in R/FR-M treatment at most sampling points during storage.

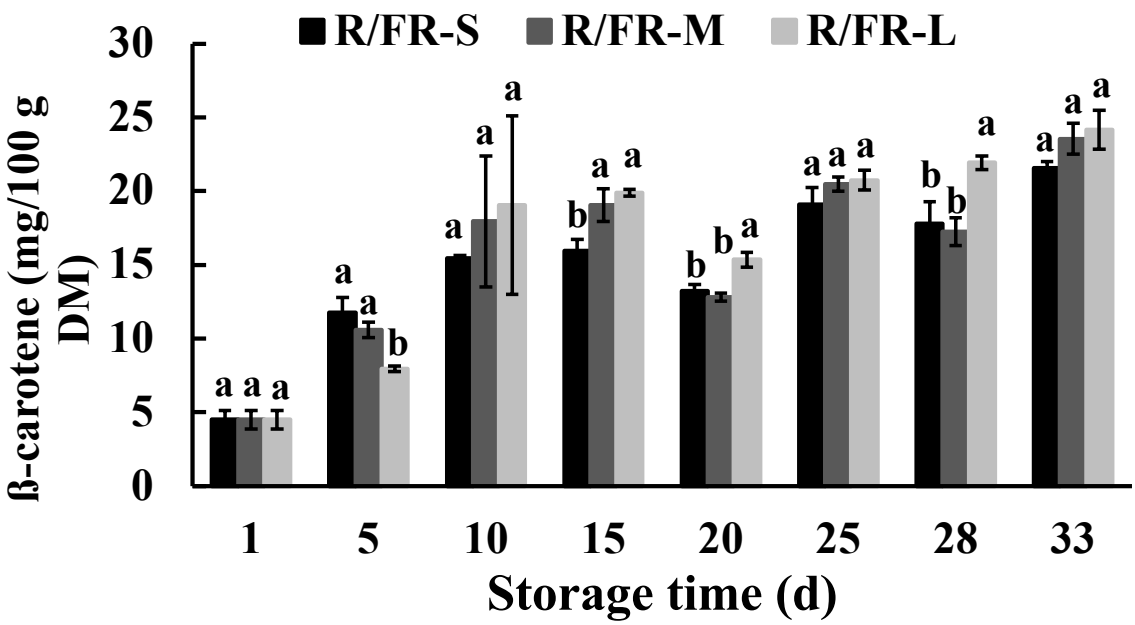


Figure 4.10 **Effects of red/far-red light irradiation time on β-carotene content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

β-carotene content was significantly affected by blue light irradiation time ($P < 0.05$) (Table 4.1). The content of β-carotene accumulated in all treatments throughout the storage time (Figure 4.11). At day 33, the highest content was found in B-M treatment (18.1 mg/100 g), followed by B-S (17.7 mg/100 g) and B-L treatment (16.7 mg/100 g). β-carotene content in B-L treatment was significantly higher than that in B-S treatment at day 10 and 20. While in R/FR-M treatment, the content was higher than that in R/FR-S treatment, and significant difference was observed at day 10. No significant difference was observed between B-M and B-L treatment.

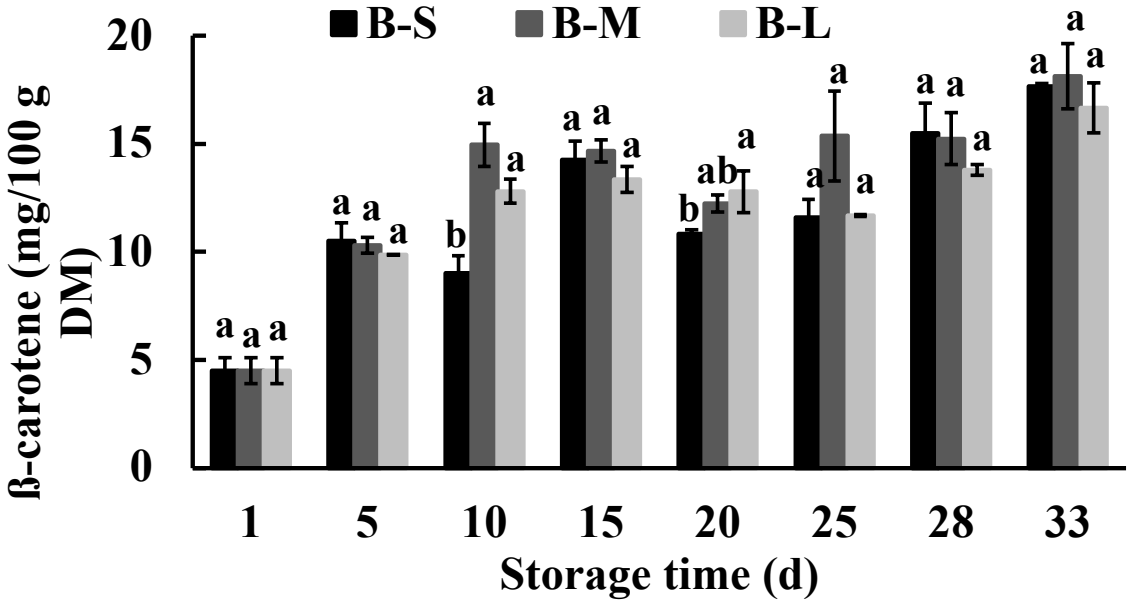


Figure 4.11 **Effects of blue light irradiation time on β-carotene content in cherry tomato fruits during storage at 15°C.** B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.6. Lutein

Lutein content was significantly affected by storage time and light treatments ($P < 0.001$) (Table 4.1). Lutein content in fruits was variable during storage (Figure 4.12). Fruits in all treatments displayed a small increase to day 5 but a gradual decline afterwards. At day 33, lutein content in fruits was 6.4, 7.5 and 5.6 mg/100 g in the red/far-red light treatment, blue light treatment and control, respectively. Fruits treated with red/far-red light had the highest lutein content from day 10 to 28, and it was significantly higher than that in control at day 20 and 28 ($P < 0.05$). The content of lutein in blue light treatment was significantly higher than that in control at day 28 and 33 ($P < 0.05$). These results show that both red/far-red and blue light inhibited the decrease of lutein during storage in comparison to control.

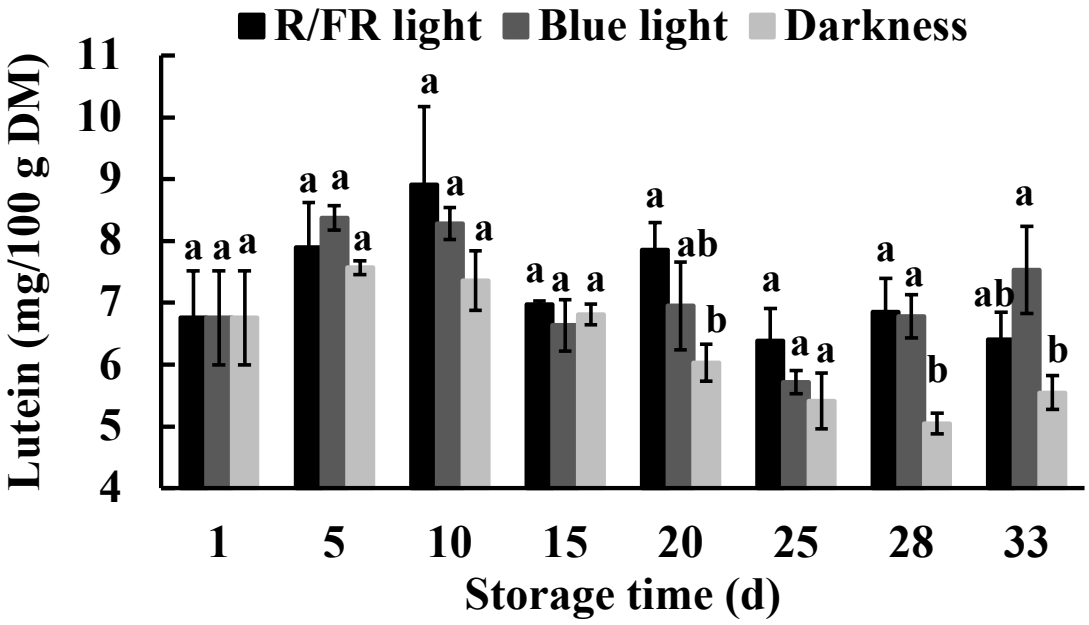


Figure 4.12 **Effects of long-duration red/far-red and blue light irradiation on lutein content in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Overall, lutein content was not significantly affected by red/far-red light irradiation time ($P < 0.05$) (Table 4.1). Lutein content in all treatments displayed a small increase to day 10 but a gradual decline afterwards (Figure 4.13). At day 33, the highest lutein content was found in R/FR-M treatment (6.5 mg/100 g), followed by R/FR-L (6.4 mg/100 g) and R/FR-S treatment (5.4 mg/100 g). The content in R/FR-L treatment was significantly higher than that in R/FR-S treatment at day 20 and 33 ($P < 0.05$). While in R/FR-M treatment, the content was significantly higher than that in R/FR-S treatment at day 33 ($P < 0.05$). No significant difference was observed between R/FR-M and R/FR-L treatment.

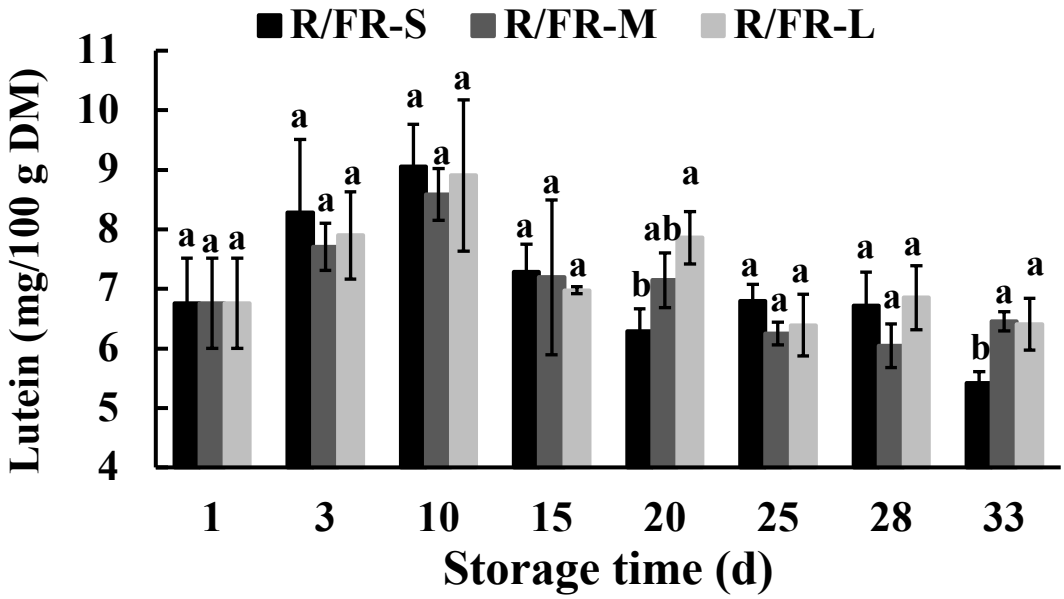


Figure 4.13 **Effects of red/far-red light irradiation time on lutein content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Overall, lutein content was not significantly affected by blue light irradiation time ($P < 0.05$) (Table 4.1). Lutein content increased slightly in all treatments at day 10 but declined afterwards (Figure 4.14). At day 33, the highest lutein content was found in B-L treatment (7.5 mg/100 g), followed by B-M (6.9 mg/100 g) and B-S treatment (6.4 mg/100 g). The content B-L, B-M treatment was higher than that in R/FR-S treatment at most days during storage, but no significant differences were observed.

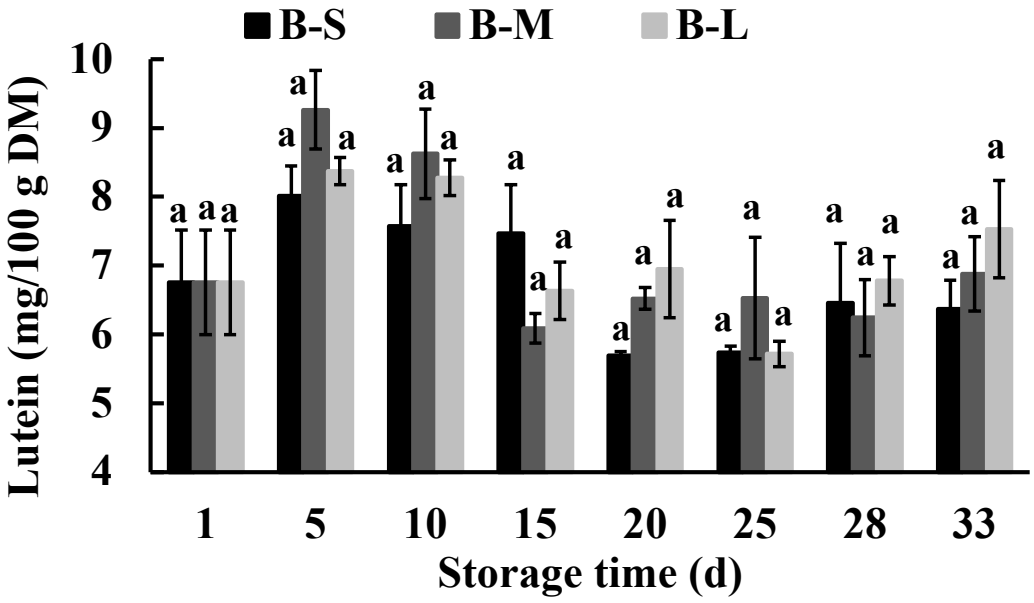


Figure 4.14 **Effects of blue light irradiation time on lutein content in cherry tomato fruits during storage at 15°C.** B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.7. Total phenolics

Total phenolics content was significantly affected by storage time ($P < 0.001$) (Table 4.1). After 33 days of storage, total phenolics content increased from 32.2 to 46.2, 36.8 and 39.4 mg/g in red/far-red light treatment, blue light treatment and control, respectively (Figure 4.15). The content in red/far-red light treatment was significantly higher than that in control at day 33 ($P < 0.05$), but no significant difference was found between blue light treatment and control.

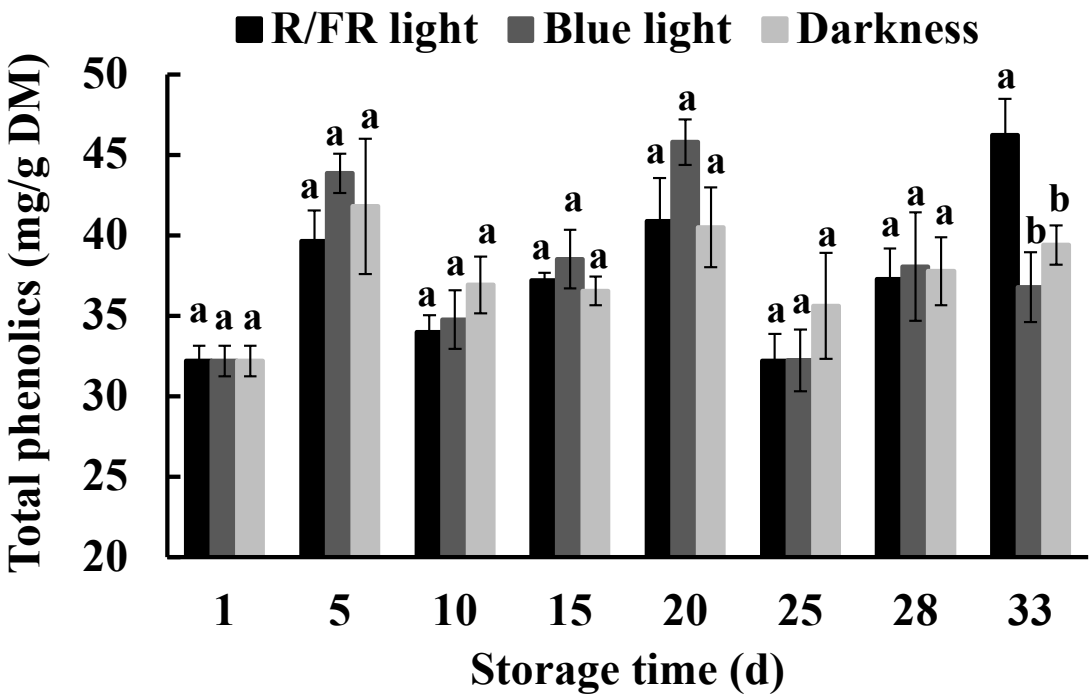


Figure 4.15 **Effects of long-duration red/far-red and blue light irradiation on total phenolics content in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Total phenolic content was significantly affected by red/far-red light irradiation time ($P < 0.05$) (Table 4.1), with the content increasing in all three treatments over the duration of the experiment (Figure 4.16). At day 33, the highest content was found in R/FR-L and R/FR-M treatment (46.2 mg/g), followed by R/FR-S treatment (41.0 mg/g). The content in R/FR-M treatment was significantly higher than that in R/FR-S treatment at day 15 and 28 ($P < 0.05$), no significant difference was found between R/FR-L and R/FR-S treatment or between R/FR-M and R/FR-L during storage.

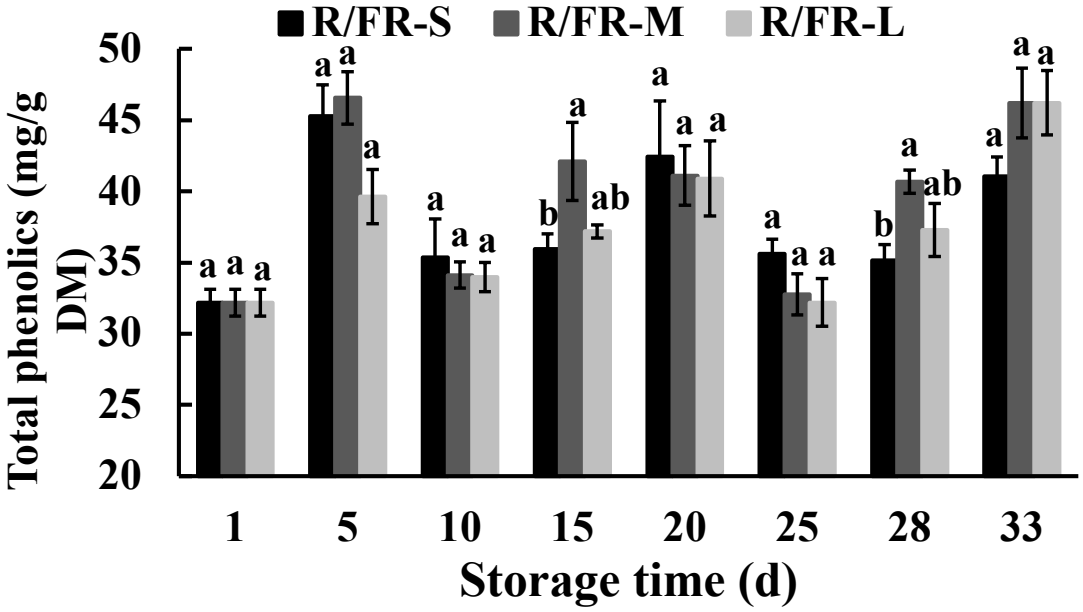


Figure 4.16 **Effects of red/far-red light irradiation time on total phenolics content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Overall, total phenolics content was not significantly affected by blue light irradiation time ($P < 0.05$) (Table 4.1). The content of total phenolics increased in all treatments during storage (Figure 4.17). At day 33, the highest total phenolics content was found in B-M treatment (41.8 mg/g), followed by B-S (39.4 mg/g) and B-L treatment (36.8 mg/g). The content in B-L treatment was significantly higher than that in B-S treatment at day 15 ($P < 0.05$). The content in R/FR-M treatment was higher than that in R/FR-S treatment at most days during storage although no significant difference was observed. There was no significant difference in total phenolics content between B-L and B-M treatment.

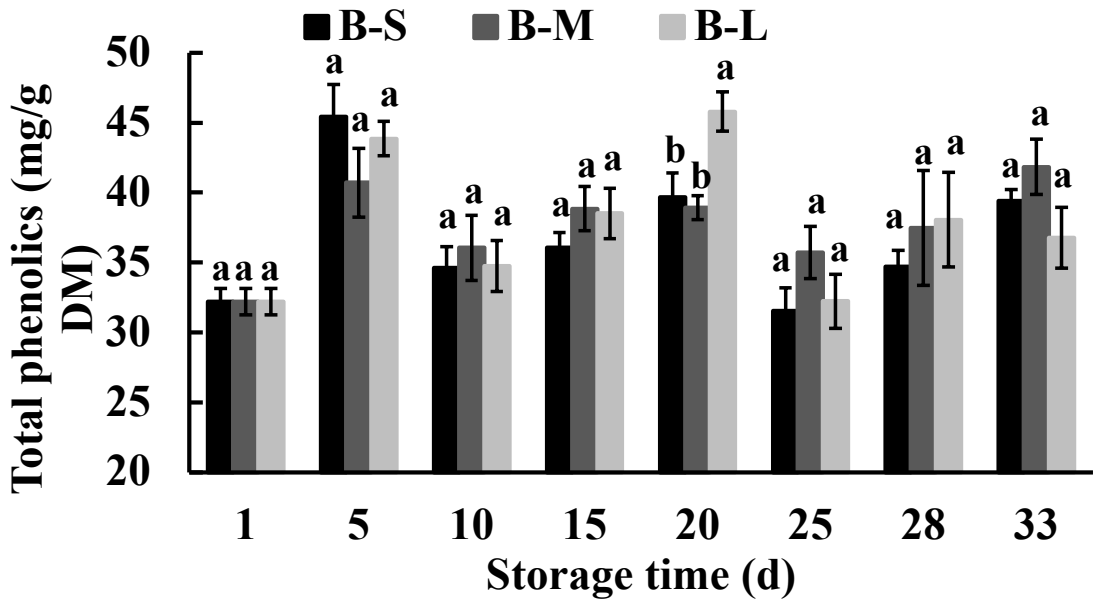


Figure 4.17 **Effects of blue light irradiation time on total phenolics content in cherry tomato fruits during storage at 15°C**. B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

4.3.8. Flavonoids

Flavonoids content was significantly affected by storage time, but not by treatments ($P < 0.001$) (Table 4.1). Generally, flavonoids content reduced over the entire storage period (Figure 4.18). At day 33, fruits treated with red/far-red light had the highest flavonoids content (8.6 mg/g), followed by blue light treatment (7.8 mg/g) and control (7.6 mg/g). The content in red/far-red light treatment was higher than that in control at most days of storage although no significant differences were found. Flavonoids content in blue light treatment was significantly higher than that in control at day 15 and 25 ($P < 0.05$).

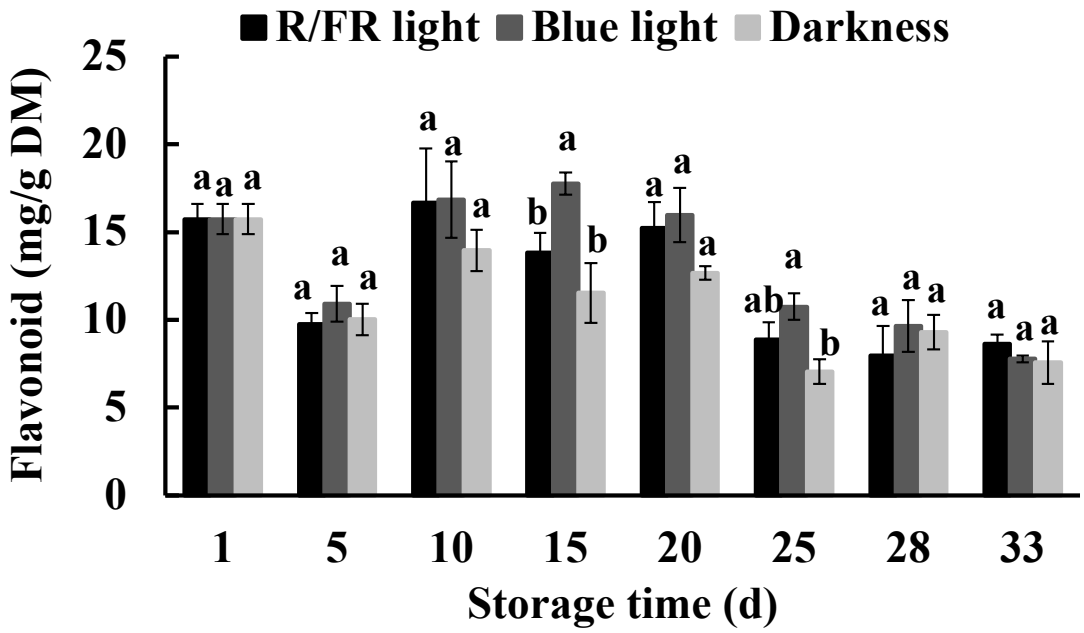


Figure 4.18 **Effects of long duration red/far-red and blue light irradiation on the content of flavonoids in cherry tomato fruits during storage at 15°C.** Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Flavonoids content was significantly affected by red/far-red light irradiation time ($P < 0.05$) (Table 4.1). The content reduced over the entire storage period in all three treatments (Figure 4.19). At day 33, the highest flavonoids content was found in R/FR-M treatment (9.5 mg/g), followed by R/FR-L (8.6 mg/g) and R/FR-S treatment (7.0 mg/g). The content in R/FR-L treatment was significantly higher than that in R/FR-S treatment at day 20 ($P < 0.05$). While in R/FR-M treatment, the content was higher than that in R/FR-S treatment during storage, and it was significantly higher than that in R/FR-S treatment at day 20 and 33. No significant difference was found between R/FR-M and R/FR-L treatment.

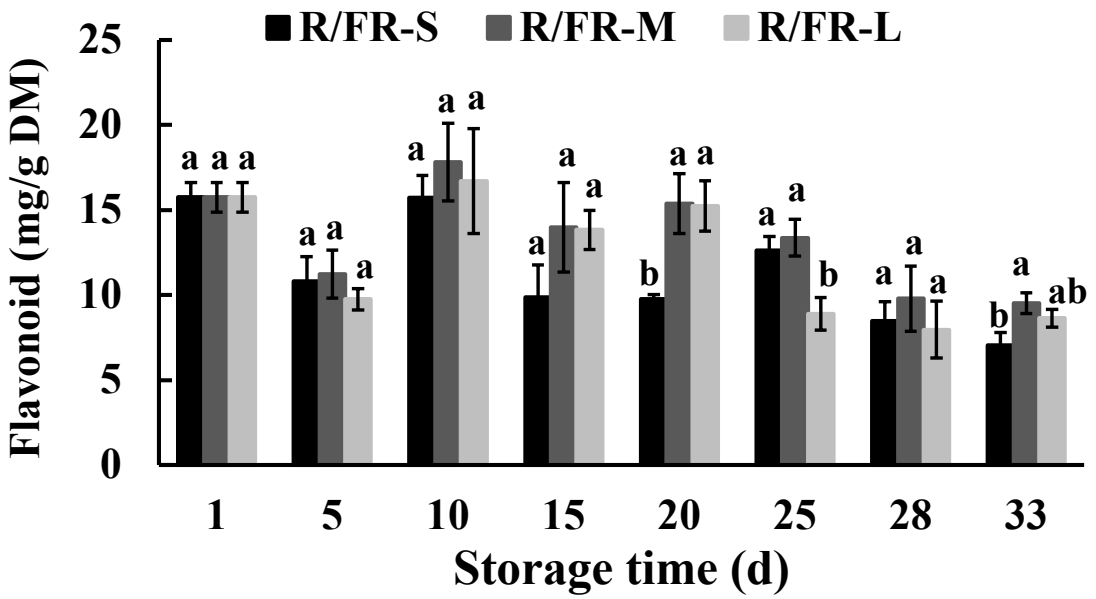


Figure 4.19 **Effects of red/far-red light irradiation time on flavonoids content in cherry tomato fruits during storage at 15°C.** R/FR-S, irradiated with red/far-red light for 5 days; R/FR-M, irradiated with red/far-red light for 15 days; R/FR-L, irradiated with red/far-red light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Flavonoids content was significantly affected by blue light irradiation time ($P < 0.05$) (Table 4.1). The content reduced over the entire storage period in all three treatments (Figure 4.20). At day 33, the highest flavonoids content was found in B-M treatment (9.1 mg/g), followed by B-L (7.8 mg/g) and B-S treatment (7.3 mg/g). The content in B-L treatment was significantly higher than that in B-S treatment at day 15 ($P < 0.05$). While in B-M treatment, the content was higher than that in B-S treatment at most days during storage, and it was significantly higher than that in B-S treatment at day 15 ($P < 0.05$). No significant difference was found between B-M and B-L treatment during storage.

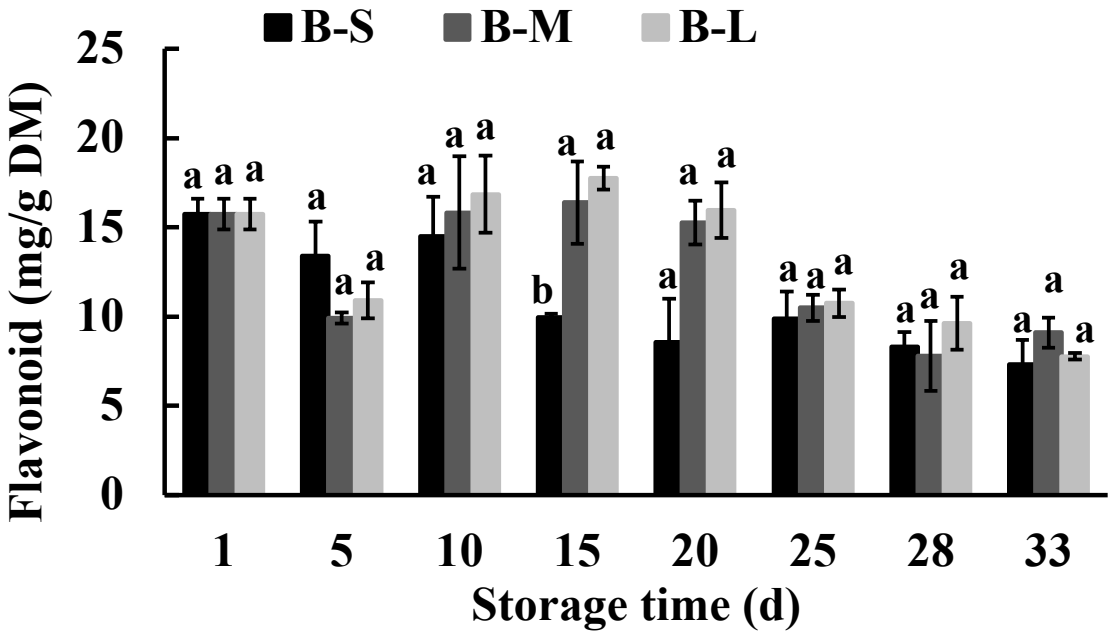


Figure 4.20 **Effects of blue light irradiation time on flavonoids content in cherry tomato fruits during storage at 15°C.** B-S, irradiated with blue light for 5 days; B-M, irradiated with blue light for 15 days; B-L, irradiated with blue light for 33 days. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Table 4.1 Analysis of nutritional quality parameters of cherry tomatoes as affected by light treatments, storage time and light irradiation time.

	Ascorbic acid	Lycopene	β-carotene	Lutein	Total phenolics	Flavonoids
Long-term light treatments (L)	*	***	***	***	ns	ns
Storage time (S)	***	***	***	***	***	***
L × S	ns	***	***	*	ns	**
Red/far-red light irradiation time	ns	*	**	ns	**	**
Blue light irradiation time	ns	***	***	ns	ns	**

*, **, *** Significant differences at $P < 0.05$, 0.01, 0.001, respectively.

'ns', not significant.

4.4. Discussion

4.4.1. Appearance

Colour turning is one of the most important and obvious changes during cherry tomato fruit ripening (Panjai et al., 2017). In this study, fruits treated with red/far-red light developed deeper red colour during storage, whereas blue light had little effect on the colour change when compared with darkness (Figure 4.1). Panjai et al. (2019), Panjai et al. (2021) and Ngcobo et al. (2021) found a similar result that red light induced colour development of tomatoes when compared with darkness. Dhakal and Baek (2014b) also found that blue light could delay colour changing from green to red in cherry tomatoes.

4.4.2. Weight loss

In this study, weight loss was observed in all three treatments, with fruits losing 3.0%, 4.3% and 3.6% of their starting weight in red/far-red light and blue light treatment, and dark control, respectively (Figure 4.2). Weight loss was reduced by red/far-red light irradiation, whereas blue light promoted it (Figure 4.2). No evidence could be found in the academic literature on the effect of red/far-red and blue light on weight loss in cherry tomatoes, although a similar result was found by Hasperué et al. (2016), who reported that Brussels sprout exposed to blue light had a higher weight loss in comparison with darkness. One possible reason could be that blue light irradiation motivates stomatal conductance and transpiration which then facilitates moisture loss during storage, while red light assists in moisture retention in vegetables and fruits (Hasan et al., 2017). But on the other hand, the light intensity differences between blue (21 μmol m^{-2} s^{-1}) and red/far-red light (10.1 μmol m^{-2} s^{-1}) used in this experiment might also had an effect on weight loss of cherry tomato fruits.

4.4.3. Ascorbic acid

4.4.3.1. The influence of storage time and light treatments on ascorbic acid content

Ascorbic acid is one of the most important nutritional components in cherry tomatoes (Fagundes et al., 2015). In this study, the results showed that when fruits were harvested at mature green stage, ascorbic acid accumulated for the first 28 days and then decreased (Figure 4.3). The same result was also found by Elwan et al. (2015), who reported that ascorbic acid content could be reduced by prolongation of storage period. The reduction may be caused by oxidizing enzymes, such as ascorbic acid oxidase, which convert ascorbic acid to dehydroascorbic acid (Albuquerque et al., 2005; Elwan et al., 2015).

Ascorbic acid content varies according to genotype, environment, storage time and condition (Raiola et al., 2014). In this study, ascorbic acid content increased from 1.6 to 2.2, 1.9 and 1.9 mg/g in the red/far-red light treatment, blue light treatment and control, respectively, after 33 days of storage (Figure 4.3). Lee et al. (2014) found that after 18 days of storage, ascorbic acid content in cabbage increased from around 0.5 to 0.7 mg/g in both red and blue light treatment, whereas it decreased slightly in darkness. The differences of content between cherry tomato and cabbage might because that they are different plant species, and their different plant types (cherry tomato is fruit while cabbage is leafy vegetable) might also be an important factor.

Red/far-red light irradiation increased ascorbic acid content when compared with darkness, whereas blue light showed little effect (Figure 4.3). A similar result was also found by Ma et al. (2014), who reported that the reduction of ascorbic acid in broccoli was delayed by red light during storage, but blue light had no effect. Lee et al. (2014) also reported that red light

increased ascorbic acid content in cabbage after 18 days of storage. The effect of red/far-red light on ascorbic acid content of tomatoes has not been reported so far.

4.4.3.2. The influence of light irradiation time on ascorbic acid content

Although after 33 days of storage, ascorbic acid content was the highest in R/FR-M (15 days' irradiation) treatment, followed by R/FR-L (33 days' irradiation) and R/FR-S (5 days' irradiation) treatment, the content was not significantly affected by red/far-red light irradiation time during storage (Figure 4.4). This suggests that compared to 15 and 33 days, 5 days' irradiation might be a better choice as it shortens light irradiation time. The similar result was found in blue light treatments (Figure 4.5). We are not aware any research about the effect of red/far-red and blue light irradiation on ascorbic acid content in cherry tomatoes.

4.4.4. Lycopene

4.4.4.1. The influence of storage time and light treatments on lycopene content

Lycopene controls redness during fruit ripening and is the most abundant carotenoid in tomatoes (Liu et al., 2011; Toor and Savage, 2006). In this study, lycopene content in cherry tomatoes increased steadily over the entire storage period (Figure 4.6), which is in accordance with the changing of colour (Figure 4.1).

Lycopene content in cherry tomatoes (cv. Piccolo) rose from 1.2 to 29.3, 28.2 and 24.7 mg/100 g in the red/far-red light treatment, blue light treatment and control, respectively, after 20 days of storage (Figure 4.6). Liu et al. (2009) reported a similar increase in red light treated Beefsteak tomatoes (cv. Red Ruby), which increased from around 2.0 to 22 mg/100 g after 21 days of storage, but in dark control, it only increased to 7.5 mg/100 g. In contrast, Panjai et al. (2017) reported a higher increase in plum tomatoes (cv. Cappricia) from 4.0 to

around 87.1 and 150.7 mg/100 g in darkness and red light treatment after 20 days of storage. The different accumulation in lycopene content might be related to the type of tomato.

In this study, red/far-red light increased lycopene content during 33 days of storage, whereas blue light had little effect on it (Figure 4.6). Schofield and Paliyath (2005) found similar result that red/far-red light increase lycopene content in tomatoes after 14 days of storage in comparison to darkness. And Dhakal and Baek (2014b) found that blue light has lower lycopene content in tomatoes than darkness.

4.4.4.2. The influence of light irradiation time on lycopene content

In this study, lycopene content increased steadily over the entire storage period in all three red/far-red light treatments. At day 33, the highest lycopene content was found in R/FR-M (15 days' irradiation) treatment, followed by R/FR-L (33 days' irradiation) and R/FR-S (5 days' irradiation) treatment (Figure 4.7). Panjai et al. (2019) reported different result in plum tomatoes that the highest lycopene content was found in tomatoes treated with red light for 20 days, followed by those treated for 15 and 10 days after 20 days of storage. Panjai et al. (2021) also reported that the highest lycopene content was found in tomatoes treated with continuous red light for 21 days, followed by those treated for 15 days and 24 hour after 21 days of storage. The different results of red light and red/far-red light might be caused by the different effects of red and far-red light on the lycopene content that red light promoted the accumulation of lycopene in tomato fruits, whereas far-red light reversed this effect (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010).

Same as red/far-red light irradiation, lycopene content in B-M (15 days' irradiation) and B-L (33 days' irradiation) treatment was higher than that in B-S (5 days' irradiation) treatment during storage, but there was no significant difference in lycopene content in all three

treatments from day 20 (Figure 4.8). We are not aware any research about the effect of blue light irradiation time on lycopene content in cherry tomatoes.

4.4.5. β-carotene

4.4.5.1. The influence of storage time and light treatments on β-carotene content

ß-carotene accumulated steadily over the entire storage time. After 33 days' storage, it had risen from 4.5 to 24.2, 16.7 and 14.4 mg/100 g in the red/far-red light treatment, blue light treatment and control, respectively (Figure 4.9). Panjai et al. (2017) reported a lower increase in plum tomatoes (cv. Cappricia), which increased from 4.4 to around 6.9 and 5.0 mg/100 g in darkness and red light treatment after 20 days of storage. The different accumulation in ß-carotene content might be related to the type of tomato.

Red/far-red light irradiation significantly promoted the accumulation of β-carotene, whereas blue light had little effect on it after day 20. Panjai et al. (2017), Panjai et al. (2019) and Panjai et al. (2021) reported similar result that red light could increase the content of β-carotene in tomatoes compared with darkness. Schofield and Paliyath (2005) also reported that red light increased β-carotene content in tomatoes after 14 days of storage in comparison to darkness. Ma et al. (2012) found that red light could enhance carotenoid content, whereas blue light had no significant influence on it in citrus fruit.

4.4.5.2. The influence of light irradiation time on β-carotene content

At day 33, R/FR-L (33 days' irradiation) treatment had the highest β-carotene content, followed by R/FR-M (15 days' irradiation) and R/FR-S (5 days' irradiation) treatment (Figure 4.10). Panjai et al. (2019) reported similar result that the highest β-carotene content was found in tomatoes treated with red light for 20 days, followed by those treated for 15 and 10 days after 20 days of storage.

β-carotene content in fruits treated with red/far-red light for 15 and 33 days was higher than those treated for 5 days during storage, and 33 days' irradiation caused to similar β-carotene content with 15 days' irradiation at most sampling days during storage (Figure 4.10). This is consistent with the finding of Panjai et al. (2021), who reported that middle- (15 days) and long-term (21 days) red light irradiation had higher β-carotene content than short-term treatment (24 hours), and the content in long-term treatment had no significant difference with that in middle-term red light irradiation.

The results for blue light are similar to that of red/far-red light irradiation as the β-carotene content in B-M (15 days' irradiation) and B-L (33 days' irradiation) treatment was higher than that in B-S (5 days' irradiation) treatment during storage, but no significant difference in β-carotene content was found in all three treatments since day 25 (Figure 4.11). This suggests that compared to 15- and 33-days' irradiation, 5 days' irradiation might be a better choice that could shorten light irradiation time.

4.4.6. Lutein

4.4.6.1. The influence of storage time and light treatments on lutein content

Fruits treated with red/far-red light had the highest lutein content at most days of storage, and the content of lutein in blue light treatment was significantly higher than that in control at day 28 and 33 (Figure 4.12), which suggests that both red/far-red and blue light inhibited the decrease of lutein during storage in comparison to control. Schofield and Paliyath (2005) found a similar result that lutein content in tomatoes treated with red/far-red light was higher than that with darkness after 14 days of storage.

4.4.6.2. The influence of light irradiation time on lutein content

At day 33, the content in both R/FR-M (15 days' irradiation) and R/FR-L (33 days' irradiation) treatment was significantly higher than that in R/FR-S (5 days' irradiation) treatment, but no significant difference was observed between 33- and 15-days' irradiation during storage (Figure 4.13). This suggests that compared to 5 and 33 days, 15 days' irradiation might be a better choice that could increase lutein content and shorten light irradiation time. The content in B-L (33 days' irradiation), B-M (15 days' irradiation) treatment was higher than that in B-S (5 days' irradiation) treatment at most days during storage, but no significant difference was observed (Figure 4.14). We are not aware any research about the effect of red/far-red and blue light irradiation time on lutein content in cherry tomatoes.

4.4.7. Total phenolics

4.4.7.1. The influence of storage time and light treatments on total phenolics content

In plants, phenolic compounds are very important secondary metabolites, and flavonoids can act as photoreceptors, antioxidants, and even antimicrobials, and they are very important nutritional components in cherry tomatoes (Fagundes et al., 2015; Liu et al., 2011). After 33 days of storage, total phenolics content in cherry tomatoes increased 44%, 16% and 22%, respectively, in red/far-red light treatment, blue light treatment and control (Figure 4.15). The increase under red/far-red light conditions is comparable to Panjai et al. (2017), who reported total phenolics content in tomatoes increased 44% in red light treatment after 20 days of storage.

The content in red/far-red light treatment was significantly higher than that in control at day 33 ($P < 0.05$), but no significant difference was found between blue light treatment and

control (Figure 4.15). Panjai et al. (2017) and Panjai et al. (2019) also reported that tomatoes treated with red light had higher total phenolic content compared to a darkness control.

4.4.7.2. The influence of light irradiation time on total phenolics content

At day 33, the highest total phenolic content was found in R/FR-L (33 days' irradiation) and R/FR-M treatment (15 days' irradiation), followed by R/FR-S treatment (5 days' irradiation) (Figure 4.16). Panjai et al. (2019) reported similar result that the highest content was found in tomatoes treated with red light for 20 days, followed by those treated for 15 and 10 days. In this study, the content in fruits treated with red/far-red light for 15 days was significantly higher than those treated for 5 days at day 15 and 28 ($P < 0.05$) (Figure 4.16). Both Panjai et al. (2019) and Panjai et al. (2021) found similar result that middle-term red light irradiation had significantly higher total phenolic content than short-term treatment.

There was no significant difference in total phenolics content in all three blue light treatment from day 25 (Figure 4.17). This suggests that compared to 15- and 33-days irradiation, 5 days' irradiation might be a better choice as it could shorten light irradiation time.

4.4.8. Flavonoids

4.4.8.1. The influence of storage time and light treatments on flavonoids content

Generally, flavonoids content in cherry tomatoes reduced over the duration of the experiment (Figure 4.18), which was in contrast to those of Panjai et al. (2017) who reported an 25% increase of flavonoids content in plum tomatoes after 20 days of storage. The different change in flavonoids content might be related to the type of tomato.

In this study, the content in red/far-red light treatment was higher than that in control at most days of storage although no significant differences were found (Figure 4.18). Panjai et al.

(2017), Panjai et al. (2019) and Panjai et al. (2021) reported similar result that the content of flavonoids in tomatoes treated with red light was higher than that with darkness during storage.

4.4.8.2. The influence of light irradiation time on flavonoids content

At day 33, the highest flavonoids content was found in R/FR-M treatment (15 days' irradiation) and R/FR-L (33 days' irradiation), followed by R/FR-S treatment (5 days' irradiation) (Figure 4.19). Panjai et al. (2019) reported that the highest content was found in tomatoes treated with red light for 20 days, followed by those treated for 15 and 10 days. In this study, the content in fruits treated with red/far-red light for 15 days was higher than those treated for 5 days during storage (Figure 4.19). Panjai et al. (2019) and Panjai et al. (2021) found similar result that middle-term red light irradiation had significantly higher flavonoids content than short-term treatment.

Although flavonoids content in B-M (15 days' irradiation) and B-L (33 days' irradiation) treatment was higher than that in B-S (5 days' irradiation) treatment at most days during storage, there was no significant difference in all three treatments at most days (Figure 4.20). This suggests that compared to 15- and 33-days' irradiation, 15 days' irradiation might be a better choice as it could shorten light irradiation time.

4.5. Conclusion

In conclusion, red/far-red light irradiation could maintain postharvest quality of cherry tomatoes by inhibiting weight loss, inducing colour changing from green to red, promoting accumulation of lycopene, β-carotene, and total phenolics content, and inhibiting reduction of lutein content compared to darkness. And in all red/far-red light irradiation treatments, 5 days' irradiation was the best choice that could keep most nutritional components of cherry

tomatoes at a relatively high level and could also shorten irradiation time. In contrast, when compared with darkness, blue light irradiation induced weight loss, and had little effect on colour change and the content of ascorbic acid, lycopene and total phenolics.

5. The combined effects of passive MAP and red/far-red light on postharvest quality of cherry tomatoes

5.1. Introduction

Modified atmosphere packaging (MAP) modifies the atmosphere within packaging. Active MAP replaces the air inside of the package with a desired gases mixture, while passive MAP uses the natural process of produce respiration, and the gas exchange ~~(respiration)~~ depending on the perforations of packaging material, to generate the modified gaseous environment (Choi et al., 2015a; Ye et al., 2012). MAP has long been used as an approach to extend shelf-life of fresh products by reducing ~~metabolic activities,~~ mechanical damage and pathological deterioration (Elwan et al., 2015; Singh et al., 2014).

Active MAP with 5% O_2 and 5% CO_2 has been applied to cherry tomatoes, and maintained firmness and changes in sugar and organic acid, decreased respiration rate and ethylene production, and reduced weight loss (Fagundes et al., 2015). Passive MAP could extend shelf-life of sugar snap peas for three weeks by maintaining visual quality, firmness, taste, chlorophyll, vitamin C and sugars contents (Elwan et al., 2015). Passive MAP has been used on cherry tomatoes, and D'Aquino et al. (2016) have found that it could preserve freshness and firmness, inhibit weight loss, and reduce degradation rate of sugars and organic acids.

Chapter 4 demonstrated that red/far-red light could maintain postharvest quality of cherry tomatoes by inhibiting weight loss and promoting nutritional quality compared to darkness. This chapter expands on that approach and considers the use of both red/far-red light and MAP on fruit quality as in grocery stores, most cherry tomatoes (such as cv. Piccolo) are packed using MAP. We used a combination of red/far-red light and passive MAP treatment to explore its effects on postharvest quality of cherry tomatoes. The effects on respiration,

appearance, weight loss and nutritional quality, including the content of lycopene, β-carotene, lutein, ascorbic acid, total phenolics and flavonoids, in fruits harvested at mature green stage and held at 15 °C for 30 days were studied, in order to explore the feasibility of replacing white light with red/far-red light in MAP condition.

The effectiveness of passive MAP is influenced by the number and diameter of perforations in the film that is used, which provides different atmosphere composition and water vapor pressures (Elwan et al., 2015; Serrano et al., 2006; Simón and Gonzalez-Fandos, 2011; Simón et al., 2008). Broccoli packaged in micro-perforated film with two holes (750 μm in diameter, one on each side of the bag) had prolonged shelf-life and reduced deterioration (Jia et al., 2009). Strawberry packaged in micro-perforated film with seven and nine holes (90 μm) maintained ~~high quality~~ firmness and total soluble solids content during storage (Kartal et al., 2012).

A pre-experiment was undertaken to explore the influence of different perforations on the concentration of O_2 and CO_2 and weight loss, to identify the optimum perforation for main experiment.

5.2. Pre-experiment

5.2.1. Methods

5.2.1.1. Treatments

Cherry tomatoes (cv. Piccolo) were harvested from a commercial greenhouse in Worcestershire, UK. Fruits were transported in a foam box to the laboratory within one hour, and then separated from the vines. Mature green fruits with uniform size, shape, as well as absence of any injury were selected, and then hand washed with tap water and air-dried at room temperature.

Fruits were divided into four treatments:

1. NPP, non-perforated package (no holes on the bags).
2. MPP15, micro-perforated package with 15 microholes (perforated with a 0.45-mm-diameter needle).
3. MPP30, micro-perforated package with 30 microholes (perforated with a 0.45-mm-diameter needle).
4. HPP, highly perforated package with 4 macroholes (0.5 cm in diameter).

For each package, eight fruits were packaged in plastic trays (volume, 716 mL) by sealing a polythene bag (length, 19.1 cm; width, 19.1 cm; thickness, 50 μm; O_2 transmission rate, 3.3×10^{-4} L m^{-2} s^{-1} MPa^{-1}; CO_2 transmission rate, 2.15×10^{-5} L m^{-2} s^{-1} MPa^{-1}). Every treatment had three packages. All packages were kept in cabinets (970, Sanyo) with constant relative humidity (85%) and temperature (15°C), and irradiated with white light for 20 days. The white light was provided by white lamp tubes (TL-D Eco 32W/840, Philips), and the light intensity was 100 μmol m^{-2} s^{-1} The concentration of O_2 and CO_2 and weight loss were determined at day 1, 5, 10, 15 and 20.

5.2.1.2. O_2 and CO_2 percentage

The same packages were used to assess headspace gas composition (% O_2 and % CO_2) at each time point, measured using a MAP-PAK O_2/CO_2 analyser (Gem Scientific, UK). Before measurement, a self-stick silicon septum was adhered to the middle of the package. The needle of the analyser was inserted through the septum to measure the O_2 and CO_2 concentration, and a trip of an adhesive tape was then placed over the septum to cover the needle hole after each measurement.

5.2.1.3. Weight loss

See 2.2 for the details.

5.2.2. Results

To understand the optimum perforations that could be applied to the combined treatment of red/far-red light and MAP, four different perforations were conducted, and O_2, CO_2 concentration and weight loss were determined.

5.2.2.1. CO_2

The CO_2 percentage was determined as percentage of headspace gas for three replicates of each treatment. During 20 days of storage, the CO_2 increased at day 5 in all treatments, and then decreased at day 15 (Figure 5.1 A). The highest CO_2 was shown in NPP (non-perforated package) treatment after 20 days of storage, with a value of 1.2%, followed by MPP15 (15 microholes) (0.3%), MPP30 (30 microholes) (0.2%) and HPP (4 macroholes) (0.03%) treatment. The CO_2 in NPP was significantly higher than the other three treatments during the whole 20 days of storage ($P < 0.05$), but no significant difference was found between the three other treatments (MPP15, MPP30 and HPP). Overall, the CO_2 concentration increased over time with a decrease in the number of perforations.

5.2.2.2. O_2

The O_2 was determined as a percentage of the headspace gas for three packages of each treatment. During 20 days of storage, the O_2 percentage, despite some divergence at day 5, remained constant in the treatment of HPP, MPP30 and MPP15 (Figure 5.1 B). O_2 in NPP treatment decreased from 18% to 16.9% at day 5, then increased to a similar value as day 1 (17.7%). A significant difference was only found at day 5 and 20 between NPP and the other three treatment ($P < 0.05$). Overall, apart from non-perforated packaging, the perforation did not affect the concentration of O_2 significantly.

5.2.2.3. Weight loss

Weight loss was determined for each treatment and expressed as the percent loss from the initial weight. Weight loss increased steadily in all treatments throughout the storage period, with the highest value found in HPP treatment at day 20 (2.0%), followed by MPP30 (1.0%), MPP15 (0.8%) and NPP (0.4%) treatment (Figure 5.1 C). During 20 days of storage, NPP had significantly the lowest weight loss ($P < 0.05$) except day 5, and then MPP15 ($P < 0.05$), MPP30 ($P < 0.05$) and NPP. Overall, the weight loss over time increased with the amount of perforations in the packaging.

Although non-perforated packaging (NPP) had the highest CO_2, the lowest O_2 and weight loss during storage, it is not recommended for the storage of fruits, as according to Almenar et al. (2007), non-perforated packaging may cause the breakdown of tissue and off flavour development on fruits because of the low concentration of O_2. As a consequence, MPP15 was chosen for the next MAP treatment, because it returned higher CO_2 and had the lowest weight loss compared to the other two treatment (MPP30 and HPP).

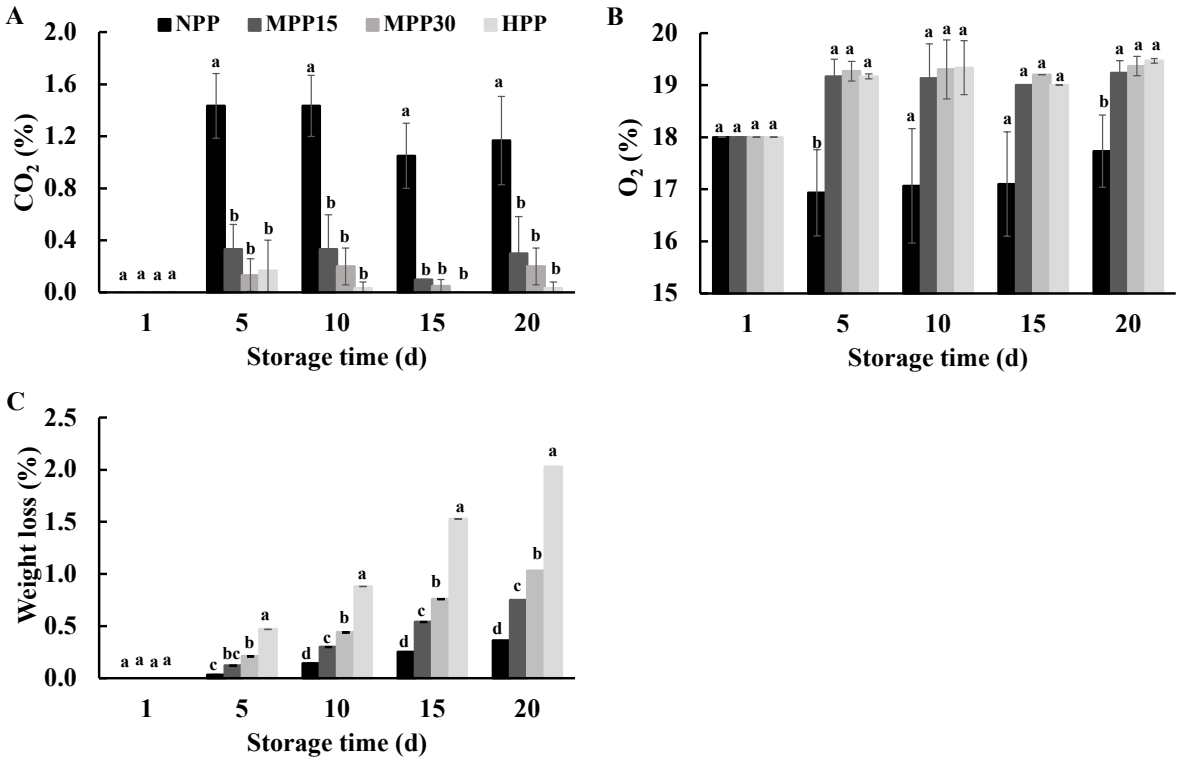


Figure 5.1 **Changes of CO_2 (A), O_2 (B) ~~concentrations~~ and weight loss (C) of cherry tomatoes inside packages in darkness.** NPP, non-perforated package. MPP15, micro-perforated package with 15 holes. MPP30, micro-perforated package with 30 holes. HPP, highly perforated package with 4 big holes. Values are the means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.2.3. Discussion

In passive MAP, the internal gas mixture and water vapor pressure are governed by the numbers and dimensions of the micro-perforations present in the packaging film; these subsequently affect the effectiveness of passive MAP and storability and shelf-life of produces (Elwan et al., 2015; Jia et al., 2009; Simón and Gonzalez-Fandos, 2011). Pre-experiment investigated four different perforations (non-perforation, NPP; 15 microholes, MPP15; 30 microholes, MPP30; 4 macroholes, HPP) for passive MAP treatment, and determined the change of O_2, CO_2 and weight loss during storage to identify their influence on gas condition and weight loss. This evidence then informed the optimum numbers of

perforations that can be applied to the combined treatment of passive MAP and red/far-red light.

In NPP treatment, CO_2 increased at day 5 and then decreased slightly till the end of storage (day 20), whereas O_2 did the opposite; it decreased to start with and then increased to day 20 (Figure 5.1 A and B). Elwan et al. (2015) reported similar result that CO_2 concentration in NPP treatment increased quickly during the first week, and then decreased by the end of storage (day 21), whereas O_2 concentration decreased during the first 7 days of storage, then increased by the end of storage. In all four treatments, NPP had the highest CO_2 and the lowest O_2 during 20 days of storage (Figure 5.1 A and B), which is consistent with the study of Elwan et al. (2015), D'Aquino et al. (2016) and Serrano et al. (2006). Although non-perforated packaging (NPP) had the highest CO_2, the lowest O_2 and weight loss during 20 days of storage, it is not recommended for the storage of cherry tomatoes, because according to D'Aquino et al. (2016), the high humidity, the presence of condensation and vapor saturated atmosphere inside of the packing lead to pathogen growth, cracking and high losses for decay, and Almenar et al. (2007) also found that non-perforated packaging may cause the breakdown of tissue and off flavour development on fruits. Therefore, to avoid the potential drawbacks of NPP on cherry tomato fruits, I decided not to choose it as MAP treatment for the main experiment.

Although no significant difference was found in CO_2 and O_2 concentration between the rest three treatments (MPP15, MPP30 and HPP), CO_2 concentration increased with the decrease of perforation (Figure 5.1 A and B), which is consistent with the study of Elwan et al. (2015) and D'Aquino et al. (2016). The weight loss increased with the perforation: MPP15 had the significant lowest weight loss in these three treatments, whereas HPP had the significant

highest weight loss during 20 days of storage (Figure 5.1 C). Elwan et al. (2015) found the similar result that HPP had the significant highest weight loss, and weight loss increased with the perforation of the packing (MPP6, MPP12 and MPP24), but no significant difference was found between micro-perforated packaging treatments. In the study of D'Aquino et al. (2016), the highest-perforated packaging (40 times) had the significant highest weight loss, whereas the rest micro-perforated had similar values of weight loss. Of the perforated treatments, MPP15 had highest CO_2 concentration that can restrict respiration rate and the lowest weight loss that extend shelf-life. High respiration leads to rapid use of its nutrients, so restricted respiration would help to preserve nutritional quality (Ye et al., 2012). Therefore, we chose MPP15 for next step's MAP treatment.

5.3. Main experiment

5.3.1. Methods

5.3.1.1. Passive MAP and light treatments

See 2.1.4 for the details.

5.3.1.2. O_2 and CO_2 concentration

See 5.2.1.2 for the details.

5.3.1.3. Respiration

Respiration was measured using the method described by D'Aquino et al. (2016). At each time point, three fruits of each package were incubated in a 0.1 L glass jar, whose lid was fitted with parafilm for 4 h. Respiration was calculated using the release of CO_2 and expressed as mg/g/h.

5.3.1.4. Appearance and weight loss

See 2.2 for the details.

5.3.1.5. Ascorbic acid

See 2.3.1 for the details.

5.3.1.6. Carotenoids (lycopene, β-carotene, and lutein)

See 2.3.2 for the details.

5.3.1.7. Total phenolics and flavonoids

See 2.3.3 for the details.

5.3.2. Results

5.3.2.1. O_2 and CO_2 ~~concentration~~

CO_2 was determined as percentage of headspace gas for MAP and R/FR-MAP but not for the control or R/FR treatment as they were not wrapped with film, which leads to CO_2 constant and the same as air during storage.

Overall, CO_2 was significantly affected by the treatments, storage time and their interaction ($P < 0.05$) (Table 5.1). CO_2 increased during the first 10 days of storage, and then decreased by the end of storage in both MAP and R/FR-MAP treatment (Figure 5.2 A). After 10 days of storage, CO_2 in MAP and R/FR-MAP treatment increased from 0 % to 0.7 % and 0.4 %, respectively, and then decreased to 0.4 % and 0.3 %, respectively, at day 30. CO_2 concentration in MAP at days 10 and 30 was significantly higher than that in R/FR-MAP treatment ($P < 0.05$).

O_2 was determined as percentage of headspace gas for MAP and R/FR-MAP but not for the control or R/FR treatment. Overall, O_2 was significantly affected by storage time ($P < 0.05$), but not by treatments (Table 5.1). O_2 decreased in both MAP and R/FR-MAP treatment during the first 20 days of storage and then increased until day 30 (Figure 5.2 B). After 20

days' storage, O_2 in MAP and R/FR-MAP treatment decreased from 19.0 % to 18.4 and 18.3 %, respectively, and then increased to 18.8% at day 30 (Figure 5.2 B).

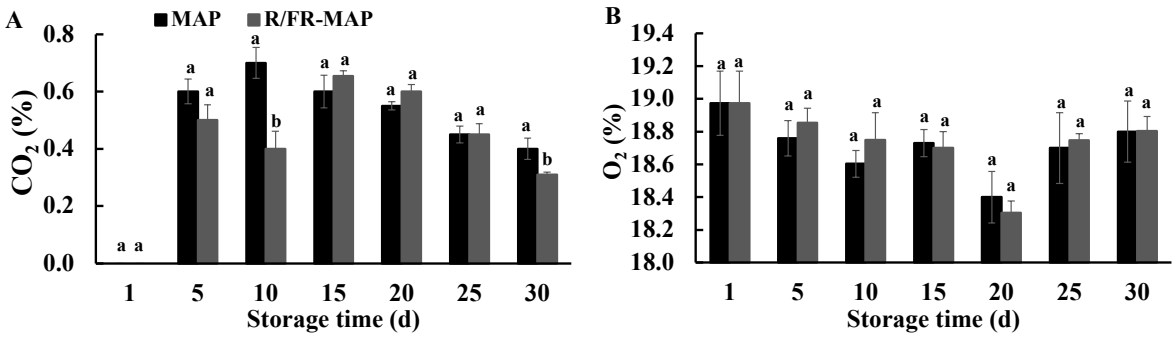


Figure 5.2 **Changes of CO_2 (A) and O_2 (B) in wrapped cherry tomato fruits with white or red/far-red light irradiation.** MAP, wrapped cherry tomatoes with white light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.2. Respiration

Overall, respiration was significantly affected by the treatments, storage time and their interaction ($P < 0.001$) (Table 5.1). Respiration increased slightly in all treatments during storage and then decreased after 30 days of storage (Figure 5.3). But the changing trends for each treatment were slightly different during the storage. In MAP treatment, respiration increased to its highest at day 5, which was earlier than the increase in control (at day 10). Whereas the increase of respiration in R/FR-MAP (day 15) and R/FR treatment (day 25) was later than that in control, indicating the predominant role of R/FR in delaying the increase of respiration. At the end of storage, R/FR treatment had the highest respiration (0.6 mg/g/h), followed by R/FR-MAP (0.4 mg/g/h), MAP (0.4 mg/g/h) treatment and control (0.3 mg/g/h), and the respiration in R/FR treatment was significantly higher than control.

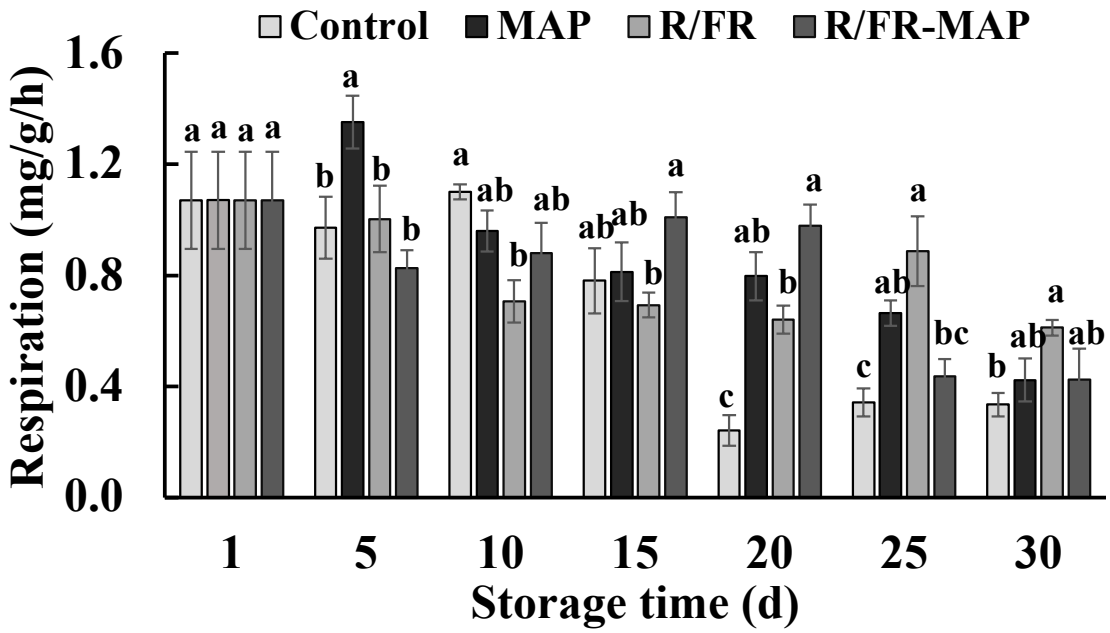
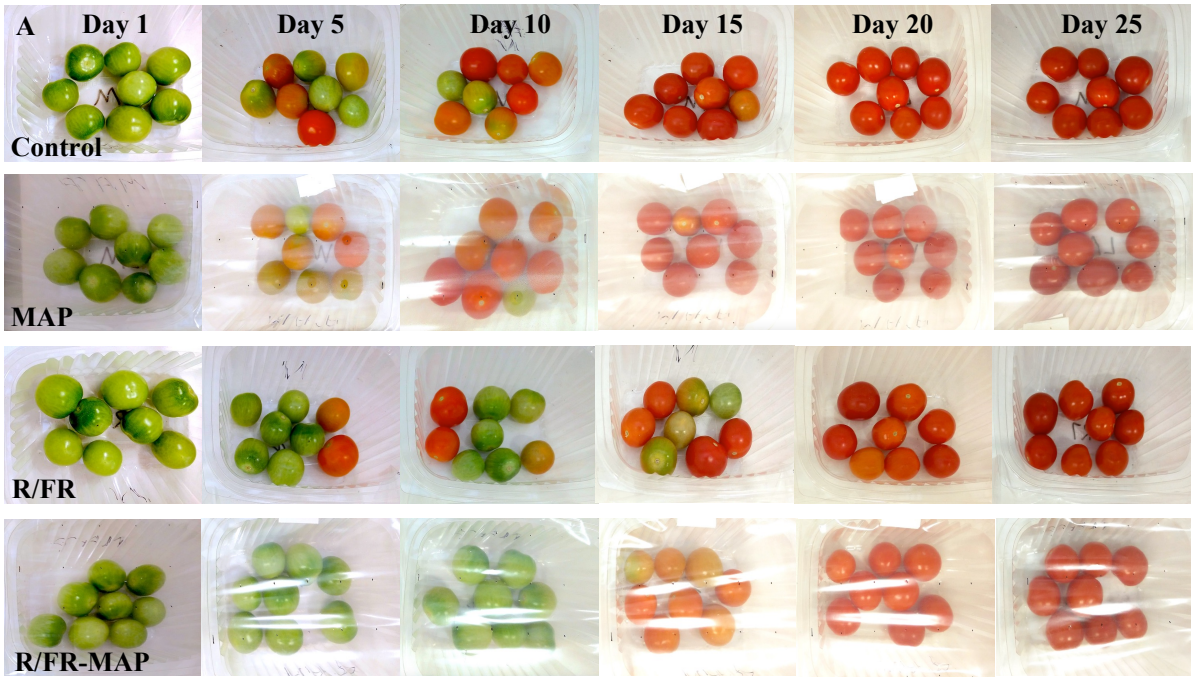


Figure 5.3 **Changes of respiration activity of wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.3. Appearance

Appearance and ripening stages were recorded using the same package at each time point. Fruit colour was affected by treatments and time (Figure 5.4 A and B). MAP treatment had more reddish (pink, light red and red) fruits than control at day 5 and 10, whereas R/FR and R/FR-MAP treatment had fewer reddish fruits than control from day 5 to 20, with R/FR treatment showing the slowest colour change process. These results show that red/far-red light delayed the ripening, as determined by colour change, of cherry tomatoes, but MAP promoted it during the first 10 days of storage.



B

	Treatments	Mature green	Turning	Pink	Light red	Red
Day 1	Control	8	0	0	0	0
	MAP	8	0	0	0	0
	R/FR	8	0	0	0	0
	R/FR-MAP	8	0	0	0	0
Day 5	Control	2	3	1	1	1
	MAP	1	3	3	0	1
	R/FR	5	1	0	1	1
	R/FR-MAP	8	0	0	0	0
Day 10	Control	1	1	3	2	1
	MAP	0	1	3	3	1
	R/FR	4	1	1	1	1
	R/FR-MAP	6	2	0	0	0
Day 15	Control	0	0	1	4	3
	MAP	0	0	1	4	3
	R/FR	1	3	2	1	1
	R/FR-MAP	0	4	3	1	0
Day 20	Control	0	0	0	4	4
	MAP	0	0	0	2	6
	R/FR	0	1	3	2	2
	R/FR-MAP	0	0	1	4	3
Day 25	Control	0	0	0	2	6
	MAP	0	0	0	1	7
	R/FR	0	0	0	4	4
	R/FR-MAP	0	0	0	3	5

Figure 5.4 Change of appearance (A) and ripening (B) in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation. Control, unwrapped

cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation.

5.3.2.4. Weight loss

Weight loss was determined for each treatment and expressed as the percent loss from the initial weight. Overall, weight loss was significantly affected by treatments, storage time and their interaction during storage ($P < 0.001$) (Table 5.1). Weight loss increased steadily in all treatments throughout the storage period, with the highest value found in control (5.0%) at day 30, followed by R/FR (4.7%), MAP (1.4%) and R/FR-MAP (1.0%) treatment (Figure 5.5). All treatments exhibited weight loss during storage, with fruits in R/FR-MAP treatment showing the lowest level. Fruits treated with MAP and R/FR-MAP exhibited significant lower values of weight loss than others ($P < 0.05$), indicating MAP mainly contributed to weight loss inhibition.

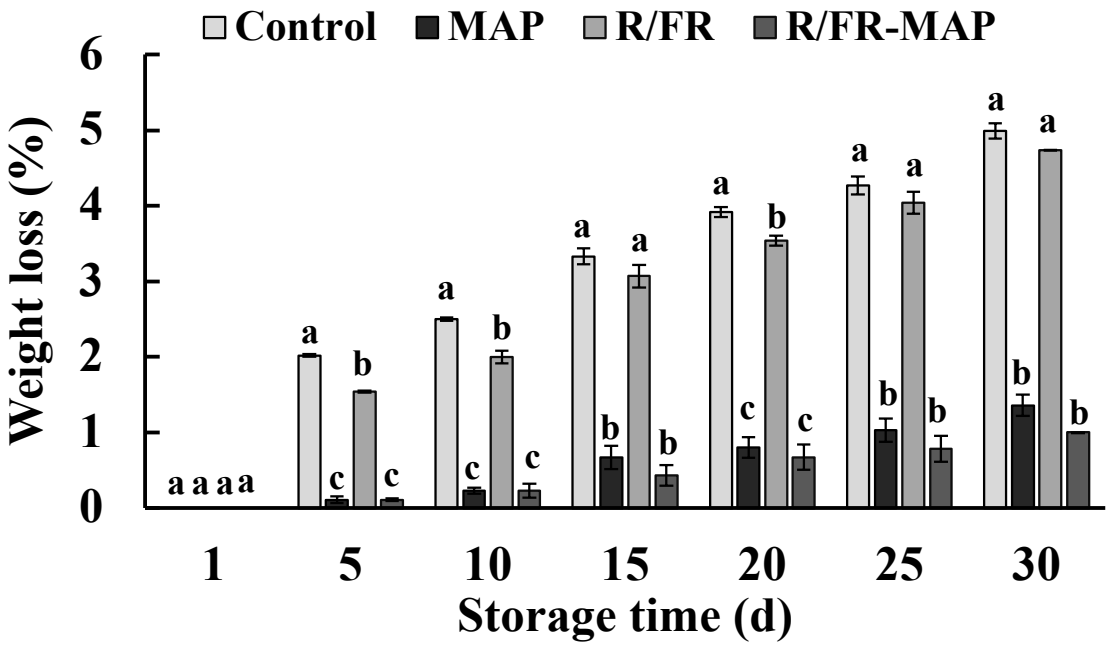


Figure 5.5 **Change of weight loss in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped

cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.5. Ascorbic acid

The content of ascorbic acid was significantly affected by storage time ($P < 0.05$) but not by treatments (Table 5.1). Ascorbic acid content dipped at day 5 and 10 across all treatments but increased thereafter (Figure 5.6). At day 30, the highest ascorbic acid content was found in control (2.2 mg/g), followed by MAP (2.1 mg/g), R/FR (1.9 mg/g) and R/FR-MAP (1.9 mg/g) treatment. The content of ascorbic acid in MAP treatment was higher than that in control from day 10 to 25, whereas in R/FR and R/FR-MAP treatment, ascorbic acid content was lower than that in control during storage, but there were no significant differences except day 25.

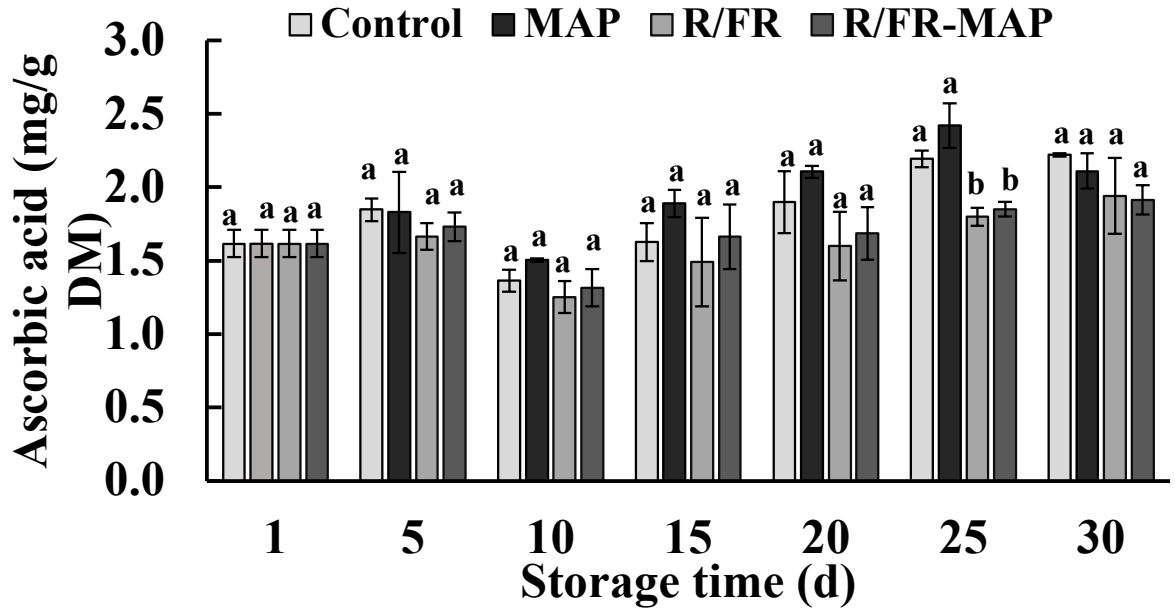


Figure 5.6 **Changes of ascorbic acid content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry

tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.6. Lycopene

The content of lycopene was significantly influenced by the treatments and storage time ($P < 0.05$) (Table 5.1). Lycopene content increased between day 15 and day 25 but declined at day 30 (Figure 5.7). At day 30, the highest lycopene content was found in MAP treatment (19.0 mg/100 g), followed by R/FR-MAP treatment (18.5 mg/100 g), R/FR treatment (17.7 mg/100 g) and control (17.4 mg/100 g). The accumulation of lycopene was induced by MAP before day 10, and it was inhibited by R/FR during the first 25 days' storage. Although there was no significant difference in the level of lycopene in R/FR-MAP relative to the control during storage, higher lycopene content was found in R/FR-MAP treatment at most days of storage.

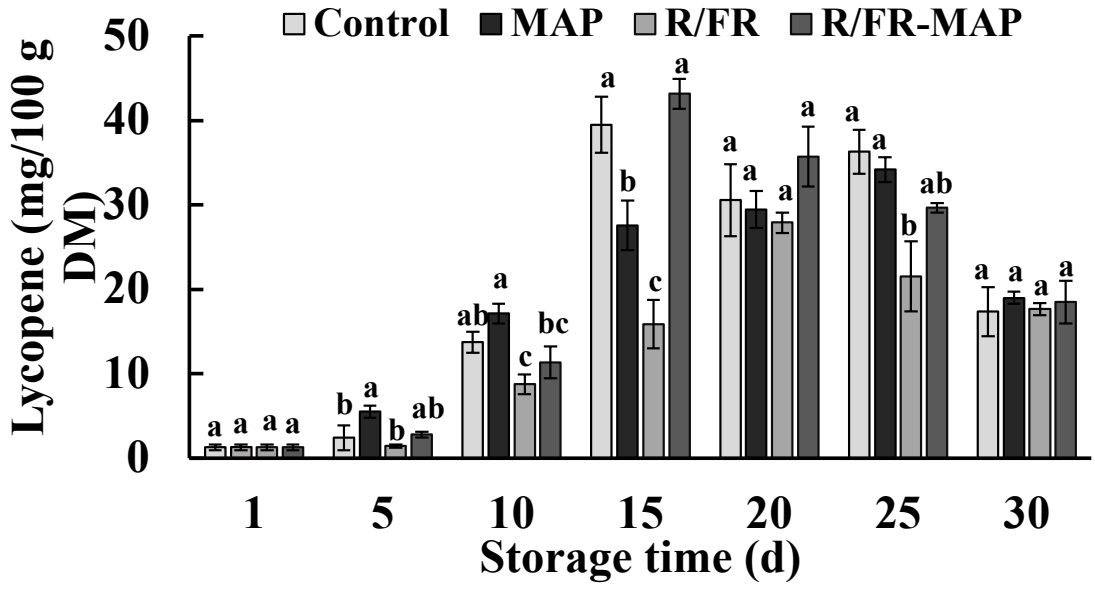


Figure 5.7 Changes of lycopene content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation. Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.7. ß-carotene

The content of ß-carotene was significantly influenced by the treatments, storage time and their interaction ($P < 0.001$) (Table 5.1). ß-carotene content increased with time up to day 20 and then remained fairly constant until day 30 (Figure 5.8). The highest content found in R/FR-MAP treatment at day 25 (22.3 mg/100 g). At day 30, the highest lycopene content was found in R/FR-MAP treatment (19.7 mg/100 g), followed by R/FR (17.7 mg/100 g), MAP treatment (17.0 mg/100 g), and control (16.2 mg/100 g). MAP and R/FR showed the reverse effect on ß-carotene accumulation in cherry tomatoes in the comparison with control, with the promotion of MAP and inhibition of R/FR. It is worth noting that ß-carotene content in R/FR-MAP treatment was higher than that in control at day 15, 25 and 30.

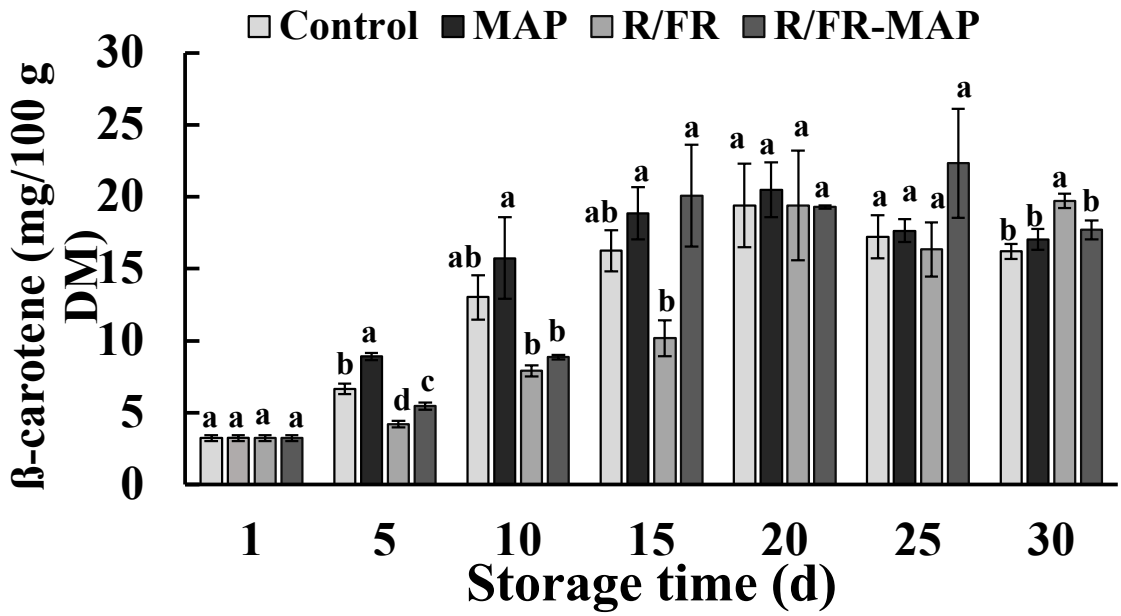


Figure 5.8 **Changes of β-carotene content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.8. Lutein

The content of lutein was also significantly influenced by the treatments, storage time and their interaction during storage ($P < 0.001$) (Table 5.1). Lutein content was elevated between day 10 and 25 but concentrations at day 30 were comparable to day 1 (Figure 5.9). At day 30, the highest lutein content was found in MAP treatment (9.1 mg/100 g), followed by control (7.2 mg/100 g), R/FR (5.7 mg/100 g) and R/FR-MAP treatment (5.3 mg/100 g). The content was significantly induced by MAP in comparison with control at day 5, 10 and 30 ($P < 0.05$). Both R/FR and R/FR-MAP treated cherry tomatoes had lower lutein content than control during storage.

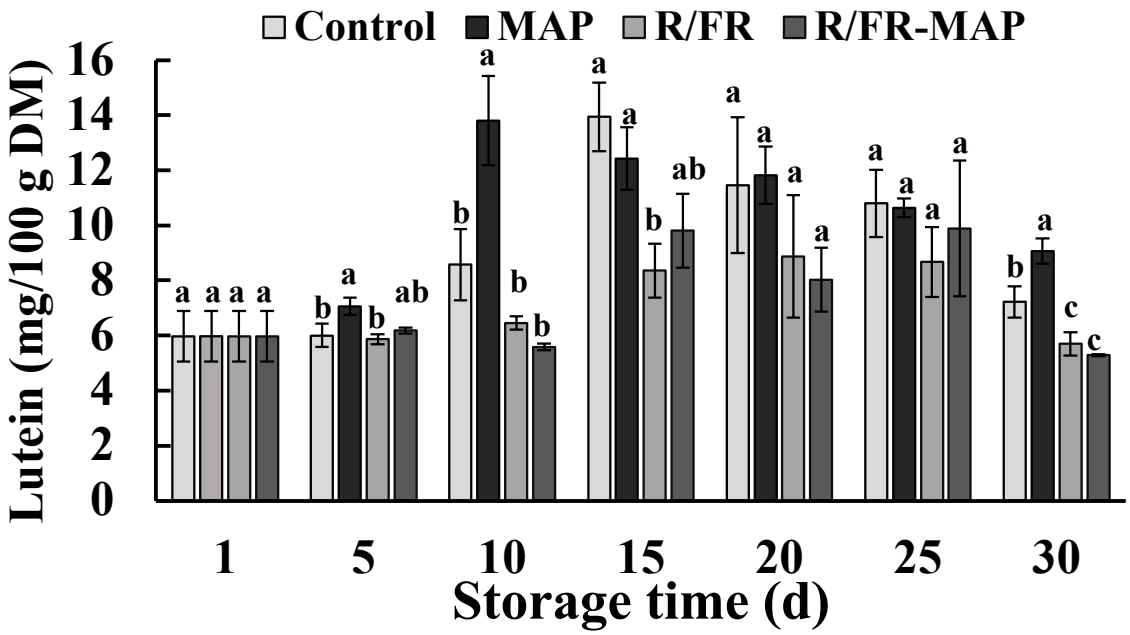


Figure 5.9 **Changes of lutein content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.9. Total phenolics

The content of total phenolics was significantly affected by the treatments and storage time ($P < 0.01$) (Table 5.1). Total phenolics content increased steadily to day 20 but then declined there afterwards to finish comparable to the day 1 value (Figure 5.10). At day 30, the highest total phenolics content was found in R/FR-MAP treatment (5.44 mg/g), followed by MAP treatment (4.72 mg/g), control (4.47 mg/g) and R/FR treatment (4.36 mg/g). The content of total phenolics in MAP treatment was higher than that in control from day 10 to 30 (Figure 5.10). The content of total phenolics in fruits treated with R/FR and R/FR-MAP was lower than that in control at the most days of storage, but no significant difference was found in R/FR-MAP relative to the control except day 5.

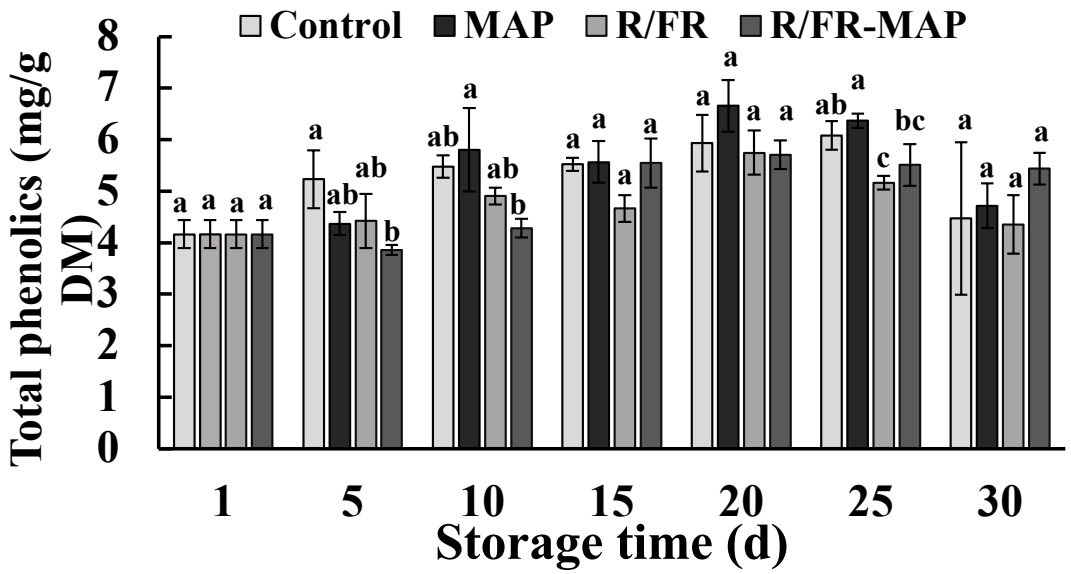


Figure 5.10 **Change of total phenolics content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

5.3.2.10. Flavonoids

The content of flavonoids was significantly affected by the treatments and storage time ($P < 0.01$) (Table 5.1) although overall concentrations were fairly stable during the experimental period. Concentrations dipped between day 5 and 25 with a slight increase at day 30 (Figure 5.11). At day 30, the highest flavonoids content was found in control and R/FR treatment (4.5 mg/g), followed by R/FR-MAP (4.4 mg/g), and MAP treatment (4.3 mg/g). The content of flavonoids in fruits treated with R/FR and R/FR-MAP was lower than that in control at the most days of storage, but no significant difference was found in R/FR-MAP relative to the control except day 5.

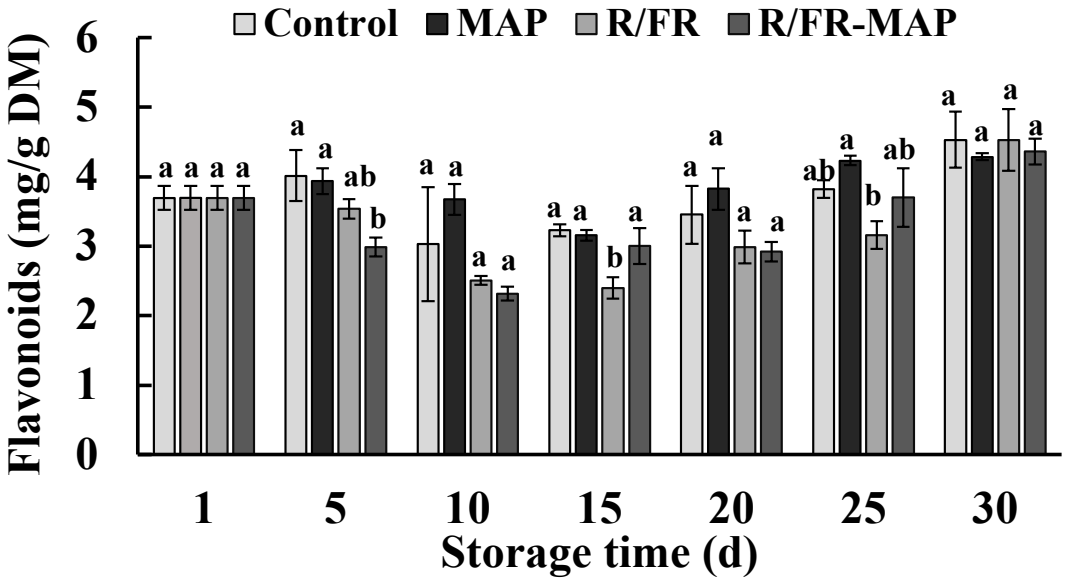


Figure 5.11 **Change of total flavonoids content in wrapped and unwrapped cherry tomato fruits with white or red/far-red light irradiation.** Control, unwrapped cherry tomatoes with white light irradiation. MAP, wrapped cherry tomatoes with white light irradiation. R/FR, unwrapped cherry tomatoes with red/far-red light irradiation. R/FR-MAP, wrapped cherry tomatoes with red/far-red light irradiation. Values are means ± SEs for triplicate samples. Different letters at the same day indicate that values are significantly different at $P < 0.05$.

Table 5.1 Analysis of the change in the atmospheric composition and quality parameters in cherry tomatoes as affected by treatments and storage time.

	CO_2	O_2	Respiration rate	Weight loss	Ascorbic acid	Lycopene	β-carotene	Lutein	Total phenolics	Flavonoids
Treatments (T)	**	ns	***	***	ns	*	***	***	**	***
Storage time (S)	***	***	***	***	***	***	***	***	***	***
L × S	***	ns	***	***	ns	ns	***	**	ns	**

5.3.3. Discussion

5.3.3.1. O_2 and CO_2 concentration

The CO_2 and O_2 concentrations were determined for MAP and R/FR-MAP but not for the control or R/FR as neither of these treatments were wrapped. CO_2 concentration increased during the first 10 days of storage, and then decreased by the end of storage in both MAP treatments, whereas O_2 concentration decreased in both MAP and R/FR-MAP treatment during the first 20 days of storage, then increased till the end of storage (Figure 5.2). Elwan et al. (2015) reported similar results that O_2 concentration in MAP treatments decreased during the first 22 days of storage, and then increased slightly, whereas CO_2 concentration showed reversed change, which might be related to the respiration of fruit inside of the packages. This is discussed further in the next section.

5.3.3.2. Respiration

The ripening of cherry tomato, as a climacteric fruit, is accompanied by an increase in respiration and ethylene production, which causes physiochemical changes and subsequently loss of quality due to texture changes, colour evolution, and aroma development (Alexander

and Grierson, 2002; Domínguez et al., 2016; Giovannucci, 2002; Mangaraj et al., 2019). In tandem with the increase of ethylene production, respiration increases sharply at the onset of ripening (Giovannoni, 2001; Majidi et al., 2014). In this study, results showed that the increase of respiration in MAP was earlier than that in control, whereas it was later in R/FR-MAP and R/FR treatment (Figure 5.3). This suggests that MAP promoted the ripening of cherry tomatoes, while R/FR delayed it. And the increase of respiration in R/FR treatment (day 25) was later than that in R/FR-MAP (day 15) (Figure 5.3). This suggests that R/FR took the predominant role in affecting the ripening of fruits. The delay of respiration and ripening caused by the combined treatment could extend shelf-life of cherry tomatoes.

The atmosphere in passive MAP is dictated by the natural respiration of products and the restricted gas exchange (Choi et al., 2015a). In this study, MAP and R/FR-MAP used the same packaging so it is assumed that they had the same gas exchange potential, therefore any differences in atmosphere are due to the respiration of products. Therefore, the significantly higher CO_2 concentration in MAP at day 10 and 30 than in R/FR-MAP treatment was caused by the significant higher respiration in MAP treatment at day 5 and 25 than that in R/FR-MAP.

5.3.3.3. Appearance

Skin colour is another important criterion that reflects the ripening process of cherry tomatoes (Panjai et al., 2017). The results showed that MAP treatment had the quickest colour changing process, whereas in R/FR and R/FR-MAP treatment, colour change was slower than in control (Figure 5.4). This is consistent with the results of respiration, indicating the predominant role of red/far-red light in delaying the ripening of cherry tomatoes. The induction of MAP on fruit colour development in this study is inconsistent with the finding of Domínguez et al. (2016), who reported that packaging delayed the development of red colour

of ‘Delizia’ cherry tomatoes, although this could be related to the red/far-red light condition in this study. In the study of D’Aquino et al. (2016), the red colour increased in both ‘Dorotea’ and ‘Trebus’ cherry tomatoes, regardless of the treatments. This suggests that the effect of MAP on fruit colour development might be related to varieties of cherry tomatoes. R/FR-MAP treatment effectively delayed fruit colour development in this study, combined with the result found above that it delayed respiration and ripening, indicating that combined treatment can extend shelf-life of cherry tomatoes.

5.3.3.4. Weight loss

In this study, a noticeable weight loss was observed in the control during storage, but the weight loss was significantly reduced in wrapping treatments (MAP and R/FR-MAP treatment) (Figure 5.5). The same results were also found in sugar snap peas (Elwan et al., 2015), shiitake mushrooms (Ye et al., 2012) and cherry tomatoes (Fagundes et al., 2015), indicating the predominant roles of MAP in inhibition of weight loss. This is likely to be due to the low water vapor transmission rate of film packaging, combined with the high respiration activity of cherry tomatoes, leading to higher relative humidity within the packaging, and hence reduced weight losses during storage (Antmann et al., 2008; Ye et al., 2012). Fruits in R/FR-MAP had had the lowest weight loss during storage (Figure 5.5), suggesting the beneficial effect of the combined treatment on storage of cherry tomatoes.

5.3.3.5. Ascorbic acid

Ascorbic acid is one of the most important antioxidants in cherry tomatoes, and it can provide protection against cardiovascular and normal cold (Ma et al., 2014; Naidu, 2003). In this study, ascorbic acid content in MAP treated fruits decreased during the first 10 days of storage after harvest, and then increased till the end of storage (Figure 5.6). This could be explained by the fact that ascorbic acid is used as a respiratory substrate (Domínguez et al., 2016)., and the increase of respiration leads to a reduction of ascorbic acid content, which

was accordance with the finding of Domínguez et al. (2016), who reported that the ascorbi acid content decreased with the increase of respiration in tomatoes.

MAP increased the content of ascorbic acid compared to control at most days of storage, but no significant differences were found. Fagundes et al. (2015) reported similar results that MAP had higher ascorbic acid than control, but no significant differences were found.

5.3.3.6. Lycopene

Carotenoids are the primary components in cherry tomato fruits, and they play important roles in reducing the incidence of some human diseases, such as cancer, heart disease and some chronic diseases (Liu et al., 2009; Rao and Rao, 2007). Lycopene (around 90%), β-carotene (5-10%) and lutein (1-5%) are the main carotenoids in ripe red tomatoes (Liu et al., 2011; Schofield and Paliyath, 2005).

Lycopene content initially increased between day 15 and day 25, but then decreased till the end of storage (day 30) (Figure 5.7), which is consistent with the finding of D'Aquino et al. (2016) and Ballester et al. (2010). The accumulation of lycopene is correlated with the colour development, as lycopene is a red pigment (Schofield and Paliyath, 2005), and the biosynthesis of lycopene in fruits is related to ripening (Alba et al., 2000a), which explains why its content increased with the ripening of fruits. Lycopene can be converted to ß-carotene by the action of LCY-b (Pandurangaiah et al., 2016), which causes the decrease of lycopene content at the end of storage. This could be supported by the evidence that ß-carotene content increased till day 30.

MAP and R/FR had reverse effects on the content of lycopene in cherry tomatoes in the comparison with control (white light), with the promotion of MAP and inhibition of R/FR

(Figure 5.7). Fagundes et al. (2015) reported similar results that MAP induced the accumulation of lycopene in cherry tomatoes compared to control. In contrast, Xie et al. (2019) compared the lycopene content of tomato fruits in red light and natural light and found that lycopene content of fruit maintained under red light treatment was higher than that under natural light during 54 days of storage. The different results of red light and red/far-red light compared to white light might be caused by the different effects of red and far-red light on the lycopene content that red light promoted the accumulation of lycopene in tomato fruits, whereas far-red light reversed this effect (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010).

The combination of passive MAP and red/far-red light effectively increased the content of lycopene at most days of storage (Figure 5.7), suggesting the dominant contribution of MAP on lycopene accumulation in the fruits with combined treatment.

5.3.3.7. ß-carotene

ß-carotene content increased during 30 days of storage (Figure 5.8), which is consistent with the findings of Xie et al. (2019), who reported that ß-carotene content in tomatoes accumulated constantly during 54 days of storage. MAP induced the accumulation of ß-carotene at most days of storage, but R/FR had reduced the content in comparison with control (Figure 5.8). Xie et al. (2019) reported that ß-carotene content in red light treatment was higher than that in natural light during 54 days of storage. The reason that why red and red/far-red light had different results compared to white light on ß-carotene content might be the same with that of lycopene, as lycopene is converted to ß-carotene with the action of LCY-B enzyme.

The combination of passive MAP and red/far-red light effectively induced the content of ß-carotene at most days of storage (Figure 5.8), indicating the dominant contribution of MAP on ß-carotene accumulation in the fruits with combined treatment.

5.3.3.8. Lutein

Lutein behaved in a similar way to lycopene, in that increased during the middle of the experimental period but then declined towards the end (Figure 5.9); the cause is likely to be the conversion of lycopene into precursor of lutein under the regulation of enzymes such as LCY-E. Xie et al. (2019) found a different result that lutein content in tomatoes treated with natural or red light decreased during storage. The content was significantly induced by MAP in comparison to control at day 5, 10 and 30, whereas both R/FR and R/FR-MAP treated cherry tomatoes had lower lutein content than control during storage (Figure 5.9), indicating the predominant role of R/FR in the control of lutein content in cherry tomatoes.

5.3.3.9. Total phenolics

Total phenolics and flavonoids are important phenolic compounds, and the health benefits of them are mainly contributed to their antioxidant activity (Heim et al., 2002). Total phenolics content increased through the first 25 days of storage and then decreased slightly at day 30 (Figure 5.10), which is consistent with Ye et al. (2012). Serrano et al. (2006) also found that total phenolics content in broccoli decreased during 28 days of storage, but Murmu and Mishra (2018) found that total phenolics content in guava decreased over 30 days. The slight difference demonstrates again that total phenolics content is species dependent.

Total phenolics was promoted by MAP at most days of storage in agreement with the finding of Murmu and Mishra (2018) and Domínguez et al., (2016). The elevation of total phenolic was inhibited ~~blocked~~ when MAP combined with red/far-red light (Figure 5.10), indicating the dominant contribution of red/far-red light.

5.3.3.10. Flavonoids

The content of flavonoids decreased in the middle of storage, and then increased slightly at the end of storage (Figure 5.11), which is consistent with Panjai et al. (2017), who reported that flavonoids in tomato fruits decreased at day 5 and then increased at the end of storage (day 20). The elevation of flavonoids was blocked when MAP combined with red/far-red light (Figure 5.11), indicating the dominant contribution of red/far-red light.

5.4. Conclusion

This was the first study to investigate the influence of the combination of passive modified atmosphere packaging and red/far-red light (0.89) on nutritional quality in postharvest cherry tomatoes. Results show that red/far-red light delayed the ripening of cherry tomatoes, as indicated by the delayed increase of respiration and slow colour change, and it inhibited weight loss. However, it reduced the nutritional quality, having lower content of lycopene, ß-carotene, lutein, ascorbic acid, total phenolics and flavonoids compared with white light. Passive MAP induced the level of ß-carotene, lutein, ascorbic acid, total phenolics and flavonoids at most times but accelerated the ripening process. Passive MAP combined red/far-red light extended shelf-life by delaying ripening and inhibiting weight loss and induced the content of lycopene and ß-carotene during storage. Although the dominant contribution of red/far-red light on the content of lutein, ascorbic acid, total phenolics and flavonoids in combined treatment was found, no significant difference was shown at most days of storage.

6. Unravelling the role and mechanism of red/far-red light in regulating lycopene synthesis in postharvest cherry tomatoes

6.1. Introduction

The biosynthetic pathway of carotenoid has been studied extensively and starts with the conversion of geranyl geranyl pyrophosphate to phytoene catalysed by phytoene synthase (*PSY*), which is the first and key-limiting step (Pandurangaiah et al., 2016; Xie et al., 2019). Phytoene is then converted into lycopene through four desaturation reactions catalysed by phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), followed by an isomerization reaction catalysed by carotenoid isomerase (CRTISO) (Pandurangaiah et al., 2016; Xie et al., 2019). Lycopene can be converted into β-carotene by the action of chloroplast lycopene beta cyclase (LCY-b), or converted into α-carotene, the precursor of lutein, by the action of lycopene epsilon cyclase (LCY-e) (Pandurangaiah et al., 2016; Rosati et al., 2000).

Light plays an important part in the biosynthesis of carotenoid (Xie et al., 2019). Red and far-red light can regulate carotenoid biosynthesis through phytochrome-mediated signalling pathways (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010). Both red and far-red light are absorbed by phytochromes (PHYs), a family of plant photoreceptors which have the ability to detect the ratio of red/far-red light (Hasan et al., 2017; Xie et al., 2019). These receptors mediate many developmental processes in plants, such as seed germination, chloroplast development, photoperiodic flowering, shade-avoidance response, and anthocyanin biosynthesis, and they also modulate the expression of light-responsive gene expression, including *PSY* (Alba et al., 2000a; Schofield and Paliyath, 2005; Toledo-Ortiz et al., 2010). Five types of phytochromes, designated phytochrome A (PHYA) to PHYE, have been founded in *Arabidopsis thaliana* (Li et al., 2011). In tomatoes, five *PHY* genes have also been identified: *PHYA*, *PHYB1*, *PHYB2*, *PHYE* and *PHYF* (Alba et al., 2000b). Among all these

phytochromes, PHYA is the primary photoreceptor responsible for perceiving and mediating responses to far-red light, whereas PHYB is the predominant phytochrome that regulates responses to red light (Li et al., 2011).

In the dark, PHYs are synthesized in their inactive Pr form, which absorbs red light, and when exposed to red light, the Pr form is converted into the active Pfr form, which absorbs far-red light (Han et al., 2007; Li et al., 2011; Quail, 1997). The active Pfr form is translocated into the nucleus and interacts with downstream signalling intermediates (such as PIFs and HY5), and transcriptionally regulates the expression of target genes (Li et al., 2011; Mereb et al., 2020; Toledo-Ortiz et al., 2010). However, the Pfr form can be converted back to the Pr from by much faster upon absorption of far-red light (Li et al., 2011; Quail, 1997). Therefore, the light quality (R-to-FR ratios) is an important factor that affects the transition between the Pr and Pfr form of phytochromes.

Phytochrome-interacting factors (PIFs) can directly interact with phytochromes, and they are negative regulators of chlorophyll and carotenoid biosynthesis (Leivar et al., 2009; Stephenson et al., 2009; Toledo-Ortiz et al., 2010). Only two members in the PIF family (PIF1 and PIF3) were reported to interact with both PHYA and PHYB (Leivar and Monte, 2014). And PIF3 is the foundation member of the PIF subset (Li et al., 2011). Long hypocotyl 5 (HY5) is a potent PIF antagonist and it has been reported that it can promote the accumulation of photosynthetic pigment in response to light (Toledo-Ortiz et al., 2014). However, all interactions identified in this phytochrome signalling pathway are protein-protein interactions (Li et al., 2011), it is not clear that if the gene expression of these transcription factors is affected by red/far-red light irradiation.

The biosynthesis of lycopene is related to ripening of fruits, which is regulated by hormones (Alba et al., 2000a; Schofield and Paliyath, 2005). The hormone ethylene is essential for the ripening of climacteric fruit, and in tomato fruits the MADS-box transcription factor ripening inhibitor (RIN) also plays a crucial role in ripening regulation (Gao et al., 2019; Yu et al., 2019). Ethylene can control fruit ripening by regulating RIN through the MADS-loop (Gao et al., 2019). In this loop, the ethylene transcription factor ethylene insensitive 3 (EIN3) binds to the promoter of RIN, and RIN binds to the promoter of the ethylene biosynthesis genes, 1-aminocyclopropane-1-carboxylate synthase 2 (ACS2) and 1-aminocyclopropane-1-carboxylic acid oxidase 1 (ACO1), which completes a positive feedback loop (Figure 6.14) (Gao et al., 2019; Lü et al., 2018). Within this loop, a small amount of ethylene can induce the expression of downstream ripening genes, including *PSY* (Gao et al., 2019). However, the mechanism of this induction and interaction between phytochrome-mediated signalling pathway and the MADS-loop in the regulation of lycopene synthesis is still not clear.

The aim of this chapter was to explore the role and mechanism of red/far-red light in regulation of lycopene biosynthesis by looking at the changes in expression levels of genes involved in carotenoid synthesis, red/far-red light receptor genes, red/far-red light transcriptional factors (TFs) genes and ripening transcriptional factors (TFs) genes.

6.2. Methods

6.2.1. Cherry tomatoes

See 2.1.1 for the details.

6.2.2. Light treatment

Fruits were divided randomly into two treatments: red/far-red light treatment and darkness (negative control). See 2.1.3 for the details of light setting up.

Each treatment contained three replicates with 24 fruits in each replicate. Three fruits were removed from each replicate at day 1, 5, 10, 15, 20, 25, 28 and 33. The fruits were immediately frozen in liquid nitrogen, and then ground into powder in liquid nitrogen and stored at -80 °C until ready for molecular analysis.

6.2.3. Carotenoids

See 2.3.2 for the details.

6.2.4. RNA extraction

See 2.4.1 for the details.

6.2.5. RNA quantification and qualification and cDNA synthesis

See 2.4.2 for the details.

6.2.6. Quantitative RT-PCR analysis

See 2.4.3 for the details.

6.2.7. Statistics analysis

SPSS Version 25.0 was used for data statistical analysis. The significant difference between samples was analysed by means of one-way ANOVA and Tukey's honestly significant difference (HSD) at a significant level of 0.05, using analysis of variance conducted according to two subjects (treatments x storage time).

6.3. Results

6.3.1. Carotenoids

A time course of post-harvest ripening was set up spanning 33 days to compare the impact of R/FR light versus dark treatment on carotenoid accumulation and the expression of a panel of key genes involved in the ripening process.

Lycopene content increased in both red/far-red light treatment and the dark control at every time point from the start to the end of the experiment (zero to day 33) (Figure 6.1). The content of lycopene in red/far-red light treatment increased significantly at day 10, 15 and 28 ($P < 0.05$), and the content at day 33 was 31 times the value at day 1 ($P < 0.05$). In the dark control, lycopene content increased significant at day 10 and 15, and the content at day 33 was 28.3 times the value at day 1 ($P < 0.05$). Red/far-red light treatment exhibited higher lycopene content than the control after day 5, and a significant difference was found between treated and untreated fruits at day 28 ($P < 0.05$).

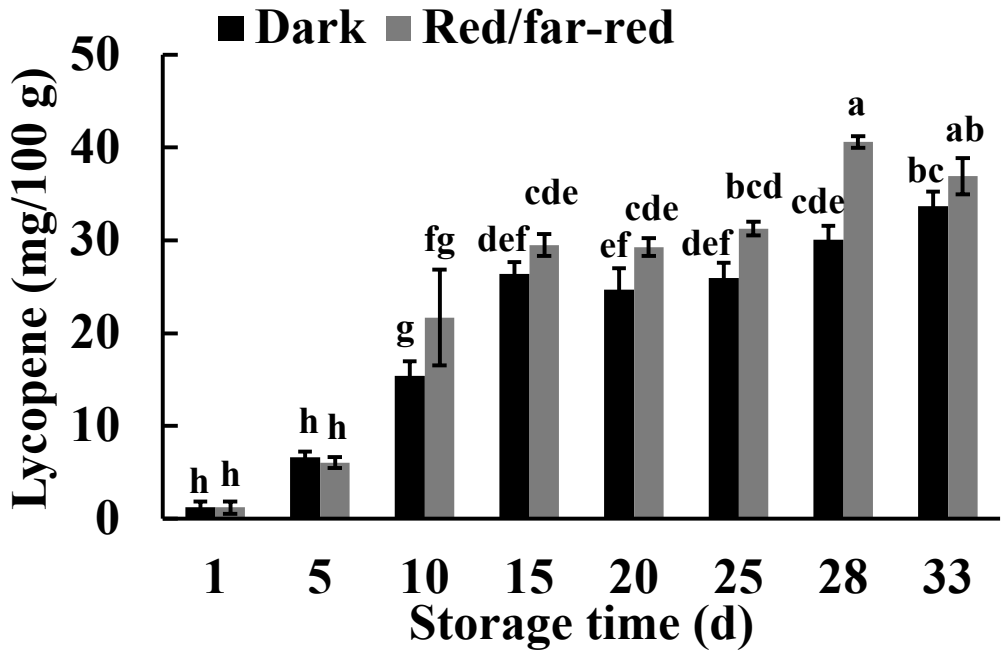


Figure 6.1 **Effects of red/far-red light irradiation on the content of lycopene in cherry tomatoes during storage.** Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

ß-carotene content increased significantly in both red/far-red light treatment and the dark control after 33 days of storage (Figure 6.2). The content of ß-carotene in red/far-red light treatment increased significantly at day 10 ($P < 0.05$), and then continued accumulating till the end of storage, corresponding to 5.4 times the value at day 1, while the control increased gradually to its highest level at day 33, which was 3.2 times the value at day 1. At every time point except day 1, ß-carotene content in red/far-red light treatment was higher than the control with significant differences from day 10 to 33 ($P < 0.05$).

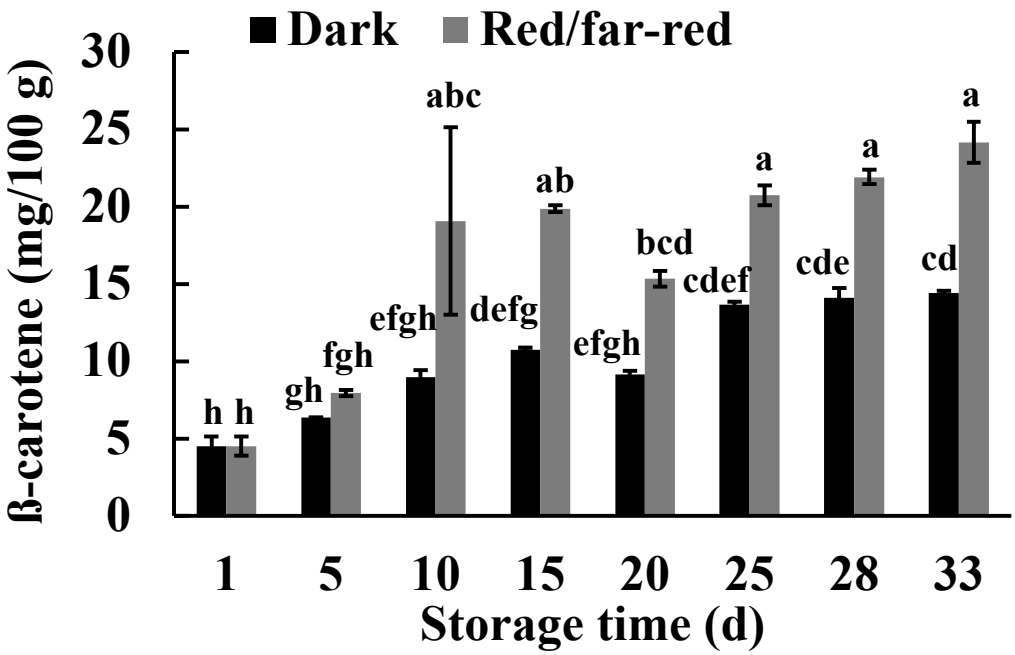


Figure 6.2 **Effects of red/far-red light irradiation on the content of β-carotene in cherry tomatoes during storage.** Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

Lutein content increased in both red/far-red light and control treatments up until day 10 but then declined afterwards (Figure 6.3). Lutein content in the red/far-red light treatment

increased 1.3~fold and reached its maximum level after 10 days of storage ($P < 0.05$) but then fell away to finish a similar level to day 1. In the control, the highest lutein content was detected at day 5, but this declined over the course of the experiment to finish at a lower level than that at day 1. At all time points, except day 1, red/far-red light treated fruits showed higher lutein content than control, although no significant differences were observed during storage.

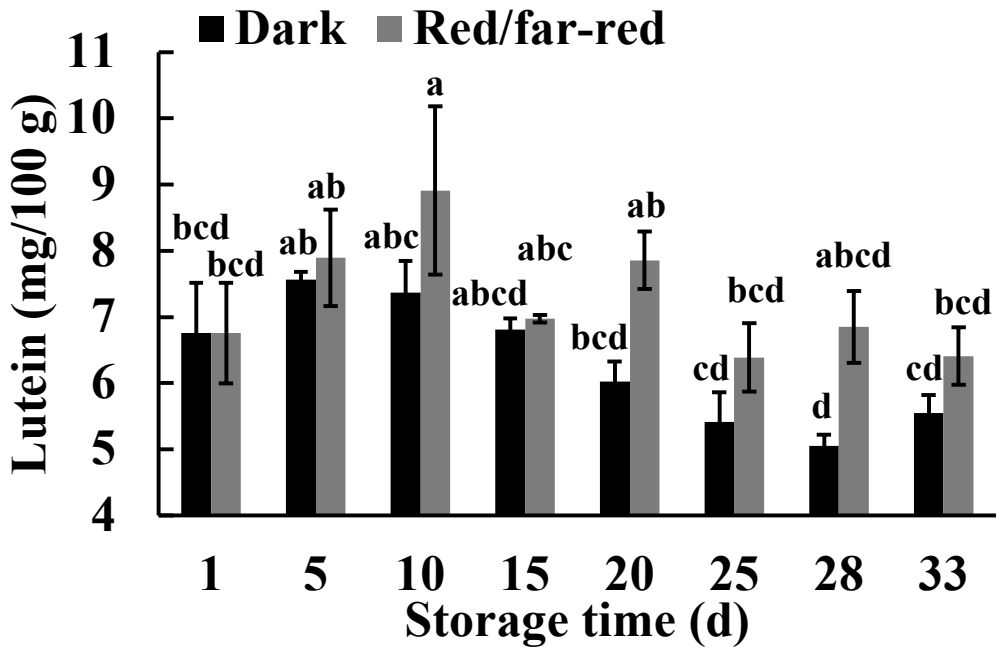


Figure 6.3 **Effects of red/far-red light irradiation on the content of lutein in cherry tomatoes during storage.** Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

6.3.2. Transcript levels of genes involved in carotenoid synthesis

PSY is the first and rate-limiting enzyme in the lycopene synthesis pathway (Pandurangaiah et al., 2016; Xie et al., 2019). The transcription of *PSY* in the red/far-red light treatment registered three peaks, which were at day 10, 20 and 28, and the accumulation of *PSY* mRNA in these days was 8.0~, 13.4~ and 9.3~fold greater than that at day 1 (Figure 6.4). In the control, the transcription level increased till day 28 (7.1~fold the initial value), and then

decreased slightly. Expression of *PSY* was higher in red/far-red light treatment than in control during storage, being noticeable the difference found at day 10 and 20 ($P < 0.05$). The results indicate that exposure to R/FR light promoted *PSY* expression and that the extent was dependent on storage time as levels of expression differed ondifferent days.

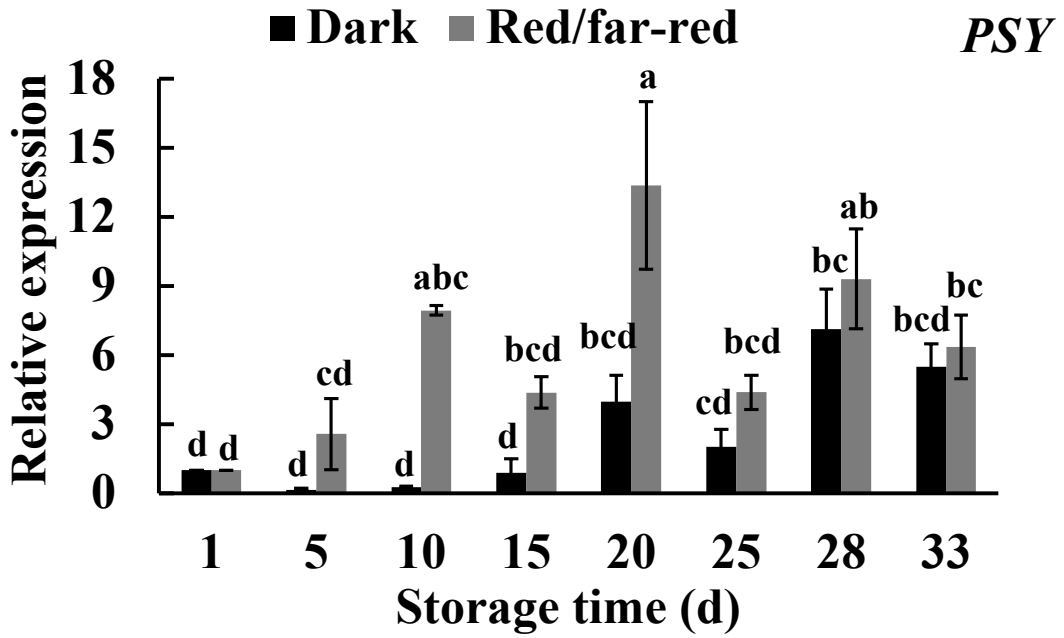


Figure 6.4 **Effects of red/far-red light on expression of lycopene biosynthesis gene *PSY* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene ***Actin***. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

ZDS is another rate-limiting enzyme in the lycopene synthesis pathway (Xie et al., 2019). The transcription pattern of *ZDS* was similar to that of PSY in that exposure to red/far-red light, registering three peaks of expression at day 10, 20 and 28, and the accumulation of *ZDS* mRNA was 5.1~, 7.1~ and 5.0~fold greater than that at day 1 (Figure 6.5). In the control, the a significant increase in level of transcript was seen on day 28 (3.1~fold the initial value), and then decreased slightly. Expression of *ZDS* was higher in red/far-red light treatment than in control during storage, a significant difference being seen at day 10, 15, 20 and 28 ($P < 0.05$).

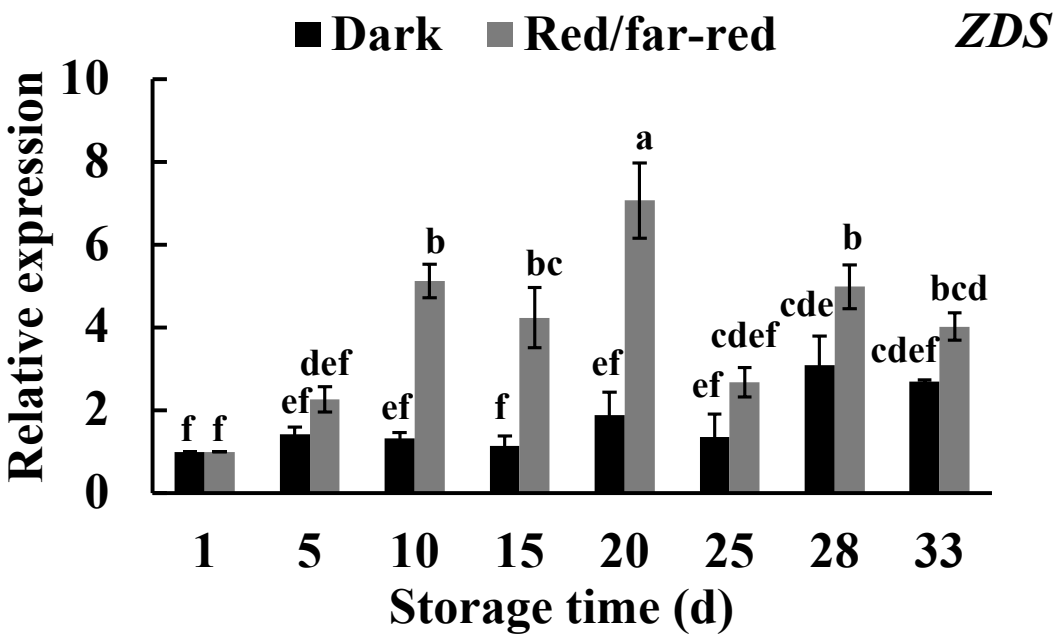


Figure 6.5 **Effects of red/far-red light on expression of lycopene biosynthesis gene *ZDS* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

LCY-b is one of the genes that mediates lycopene metabolism, and converts lycopene to ß-carotene (Fig. 6.14) (Pandurangaiah et al., 2016; Xie et al., 2019). Expression of *LCY-b* in both control and R/FR treated fruits increased significantly to a maximum after 5 days of storage (5.8~ and 2.3~fold the initial value, respectively) ($P < 0.05$), and this rise was followed by a slow decline (Figure 6.6). At day 33, the expression level of *LCY-b* in red/far-red light treatment and the control was increased by 3.5 and 1.1~fold, respectively, in comparison to the value at day 1. The red/far-red light treatment showed significantly higher expression of *LCY-b* gene than control at each time point analysed except day 1, 15 and 25 ($P < 0.05$).

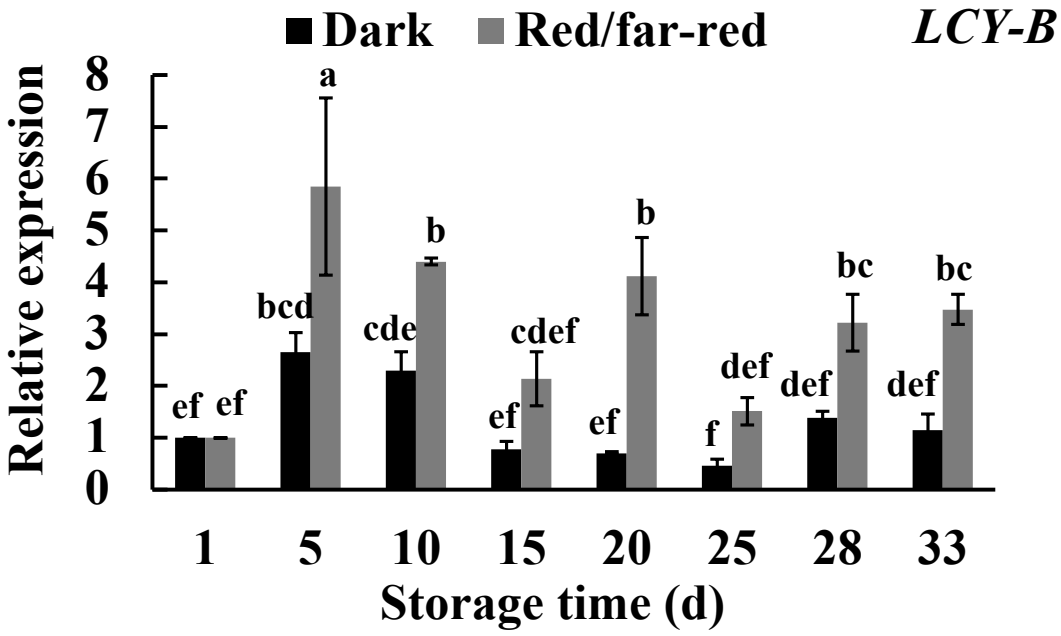


Figure 6.6 **Effects of red/far-red light on expression of lycopene metabolism gene *LCY-B* in cherry tomatoes during 33 days' storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

LCY-e is another gene that mediates lycopene metabolism, and the enzyme LCY-e converts lycopene into a-carotene, the precursor of lutein (Fig 6.14) (Pandurangaiah et al., 2016; Xie et al., 2019). The expression of *LCY-e* in both treated and untreated fruits increased significantly to their maximum after 5 days of storage (12.9~ and 3.7~fold the initial value, respectively) ($P < 0.05$) (Figure 6.7). The rapid increase expression was followed by a near 3~fold decline at day 10 in red/far-red light treatment, and the expression in both treated and untreated fruits continued decreasing to values that were lower than initial value from day 15. Expression of *LCY-e* was higher in red/far-red light treatment than in control at most days of storage, but significant difference was only found at day 5 ($P < 0.05$), and the level was 3.2~fold than that in control.

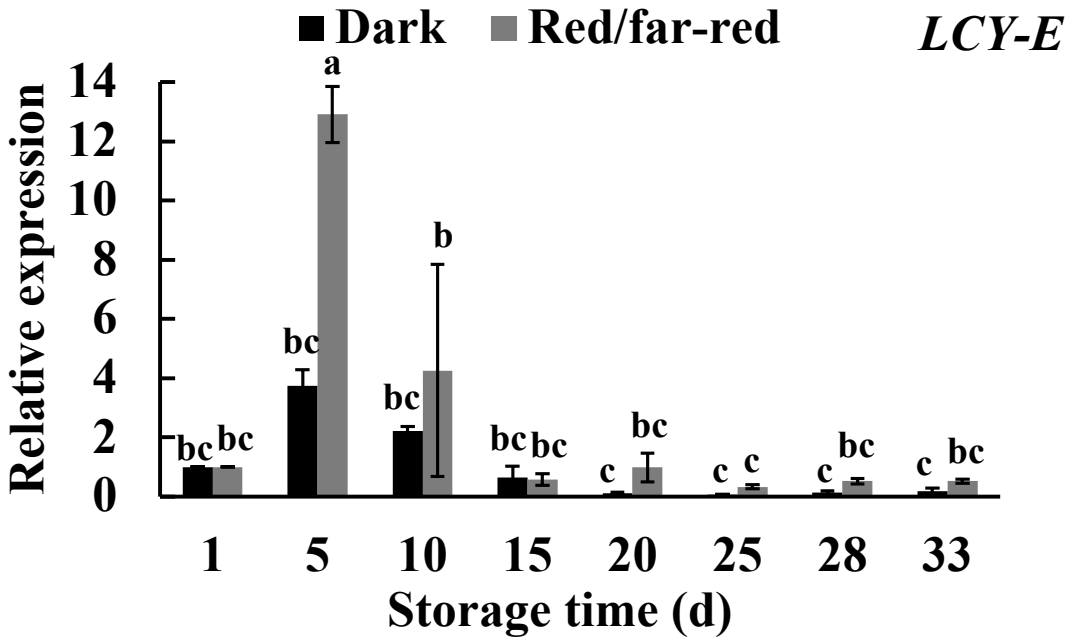


Figure 6.7 **Effects of red/far-red light on expression of lycopene metabolism gene *LCY-E* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

6.3.3. Transcript levels of genes involved in light signalling pathway

PHYB and PHYA can perceive red and far-red light, respectively, and transduce light signals to downstream signalling intermediates (Li et al., 2011; Toledo-Ortiz et al., 2010). The expression of *PHYA* in treated fruits increased significantly to its maximum after 10 days of storage (3.5~fold the initial value) ($P < 0.05$), and then decreased slightly at day 33 (2.1~fold the initial value) (Figure 6.8). Whereas in the dark control, the expression of *PHYA* increased during storage, and the highest level was shown at the end of storage (2.7~fold the initial value). *PHYA* showed higher expression level in red/far-red light treatment than in control during 28 days of storage, a significant difference was only found at day 10 ($P < 0.05$).

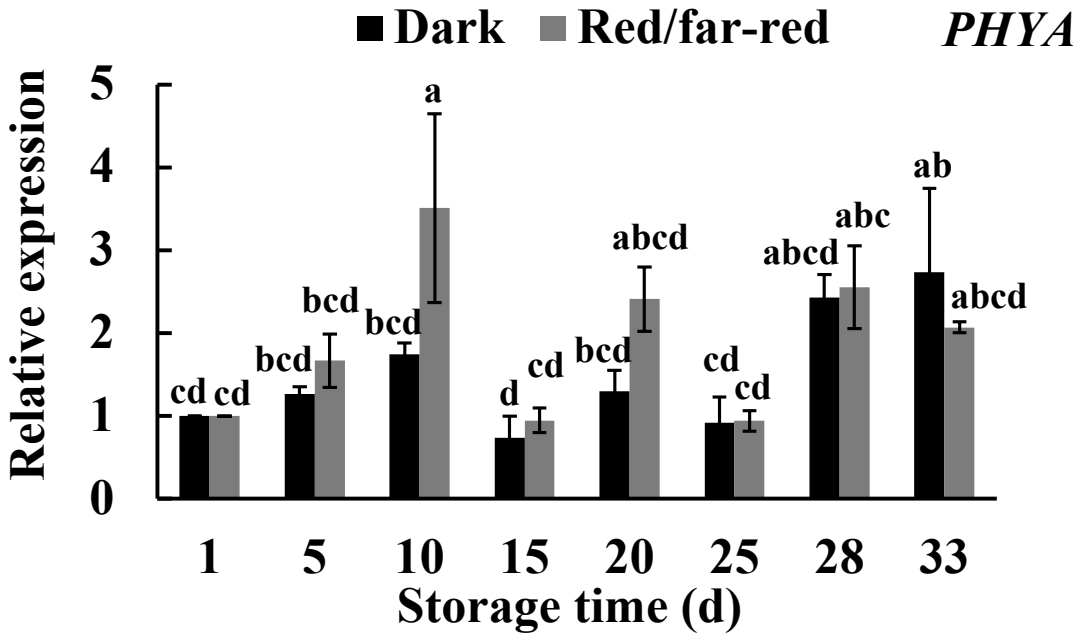


Figure 6.8 **Effects of red/far-red light on expression of light receptor gene *PHYA* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

The transcription trend of *PHYB* was comparable with that of *PHYA* during storage. The expression of *PHYB* in treated fruits increased significantly to a maximum after 10 days of storage (8.0~fold the initial value) ($P < 0.05$), and then decreased till the end of storage (day 33) (3.5~fold the initial value) (Figure 6.9). Whereas in control, the expression of *PHYA* increased during storage, and the highest level was shown at the end of storage (7.3~fold the initial value). Although *PHYB* expression level was higher in red/far-red light treatment than in control at day 5, 10 and 20, no obvious difference was found between treated and untreated fruits.

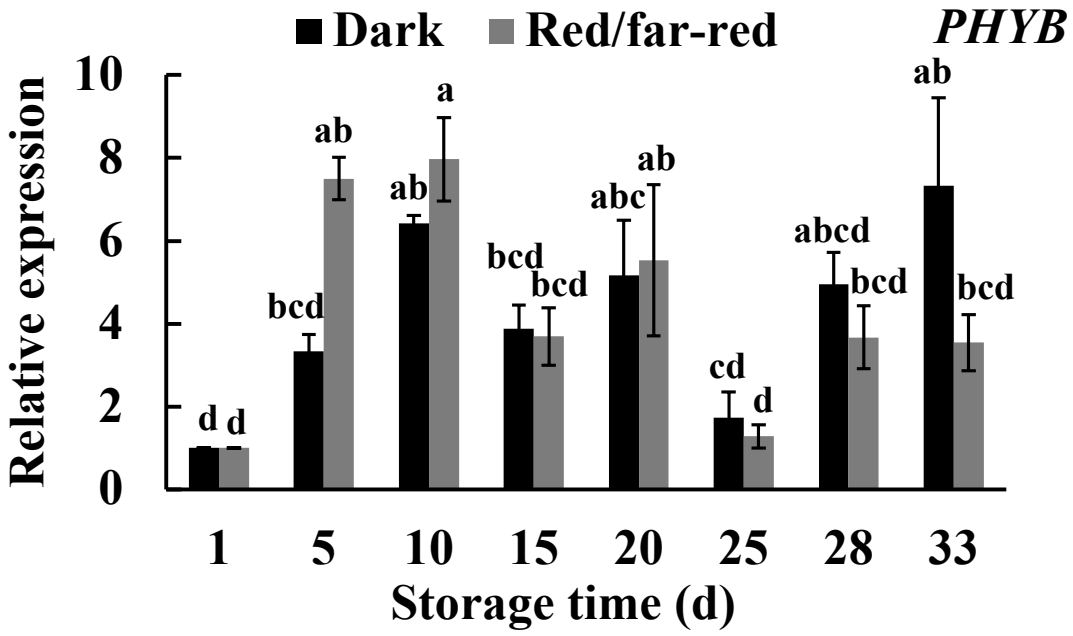


Figure 6.9 **Effects of red/far-red light on expression of light receptor gene *PHYB* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

PIF3 is the foundation member of the PIF family of genes that can interact with both PHYA and PHYB (Leivar and Monte, 2014; Li et al., 2011). The expression of *PIF3* in treated fruits increased significantly to its maximum after 5 days of storage (2.4~fold the initial value) ($P < 0.05$), and then decreased slightly at day 33 (Figure 6.10). In control, two peaks were found in the *PIF3* transcription, which were at day 10 (2.0~fold the initial value) and 28 (1.9~fold the initial value). The transcription level of *PIF3* was higher in red/far-red light treatment than in control during the first 28 days, but no obvious difference was found during storage.

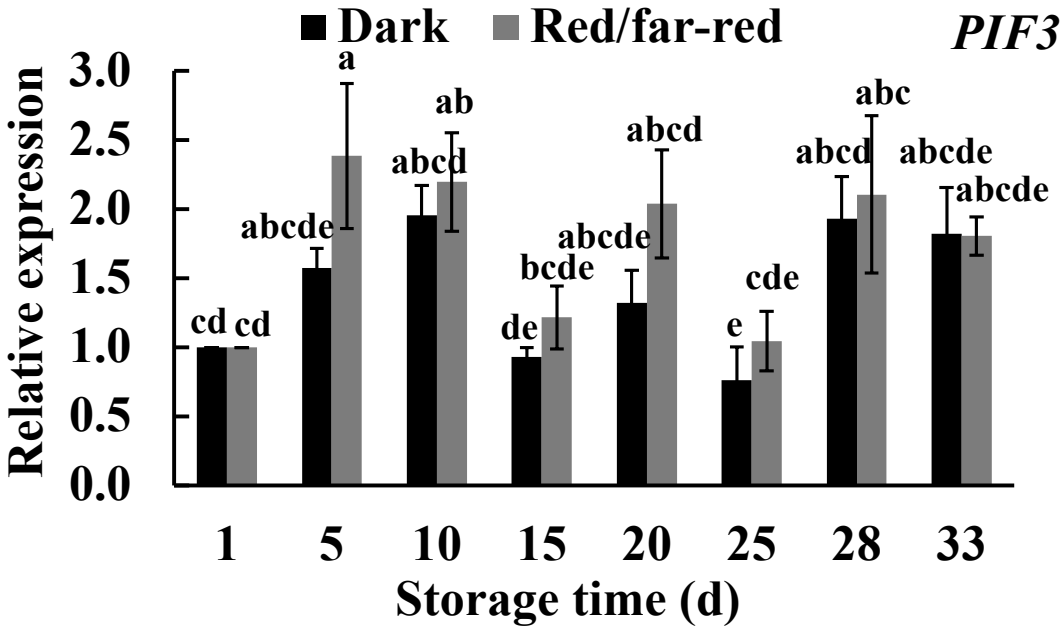


Figure 6.10 **Effects of red/far-red light on expression of light interaction transcription factor gene *PIF3* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

The expression of light signalling component *HY5* in red/far-red light treatment showed a similar trend as *PSY* during postharvest storage, registering three peaks of expression at day 10, 20 and 28, and the transcription level in these days was 1.3~, 1.9~ and 1.5~fold greater than that at day 1 (Figure 6.11). In control, the expression diminished from day 5. *HY5* level was higher in treated fruits than in untreated fruits during storage, with significant difference being noticed at day 10 and 20 ($P < 0.05$).

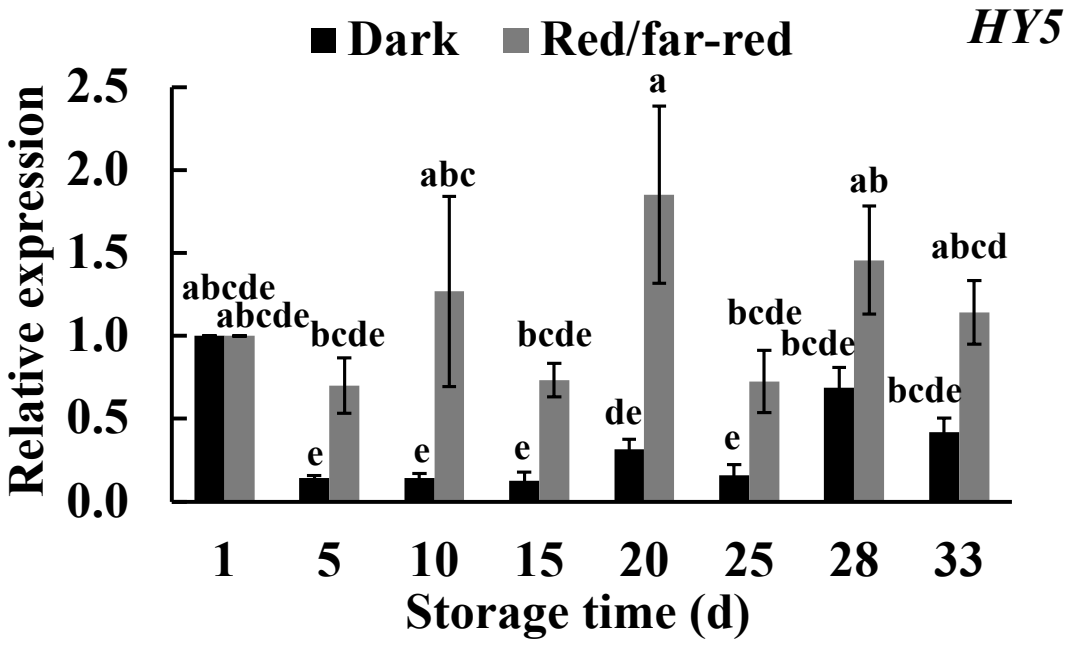


Figure 6.11 **Effects of red/far-red light on expression of light interaction transcription factor gene *HY5* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

6.3.4. Transcript levels of gene involved in MADS-loop

A burst of ethylene provides a key regulatory signal for fruit ripening phenomena, including accumulation of carotenoid (Ito et al., 2017). ACS is a rate-limiting enzyme in ethylene biosynthesis, and it catalyses S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1 carboxylic acid (ACC), the precursor of ethylene (Barry et al., 2000; Ito et al., 2017). During tomato fruit ripening, ethylene production is driven by genes *ACO1*, *ACO2*, *ACS4* and *ACS2*, while *ACS4* and *ACS2* are the two main genes (Barry et al., 2000; Ito et al., 2017; Liu et al. 2015). In tomato, A*CS2* is involved in the MADS-loop (Gao et al., 2019).

The expression of *ACS2* in red/far-red light treatment increased 19.2~fold the initial value after 10 days of storage, and 29.3~fold after 20 days ($P < 0.05$), and it decreased slightly

afterwards (Figure 6.12). At day 33, the expression level was 27.9~fold the initial value. In control, the transcription level of *ACS2* increased till the end of storage, with the value 44.2~fold the initial value ($P < 0.05$). The transcription in red/far-red light treatment was higher than that in control during the first 20 days, although no difference was found at day 25. The transcription trend of *ACS2* in red/far-red light treatment was quite similar with that of *HY5* during the first 25 days of storage.

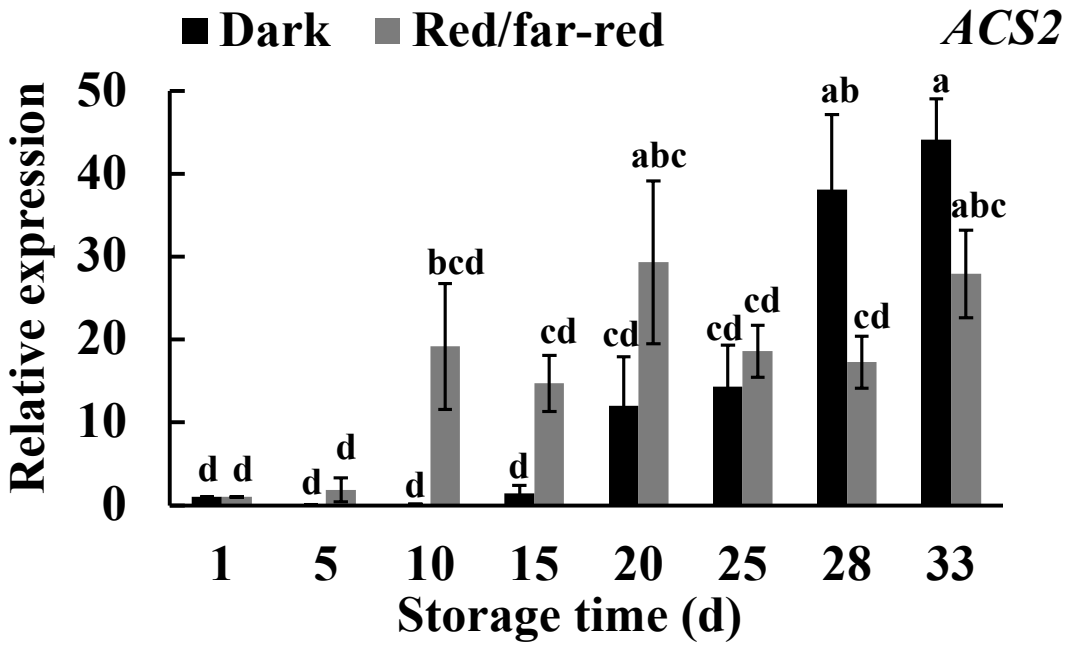


Figure 6.12 **Effects of red/far-red light on expression of ethylene biosynthesis gene *ACS2* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

RIN is one of the ripening transcription factors involved in tomato ripening and it is a key regulator in the MADS-loop (Gao et al., 2019; Ito et al., 2017). The expression of *RIN* in red/far-red light treatment showed a similar trend as *PSY* and *ZDS* during postharvest storage, registering three peaks of expression at day 10, 20 and 28, and the transcription level in these days was 13.7~ ($P < 0.05$), 16.2~ ($P < 0.05$) and 13.4~fold ($P < 0.05$) greater than that at day

1 (Figure 6.13). In control, the transcription level increased till the end of storage, with the value 15.0~fold the initial value ($P < 0.05$). Expression of *RIN* was higher in treated fruits than in untreated fruits during 28 days of storage, with a significant difference being noticed at day 10 ($P < 0.05$).

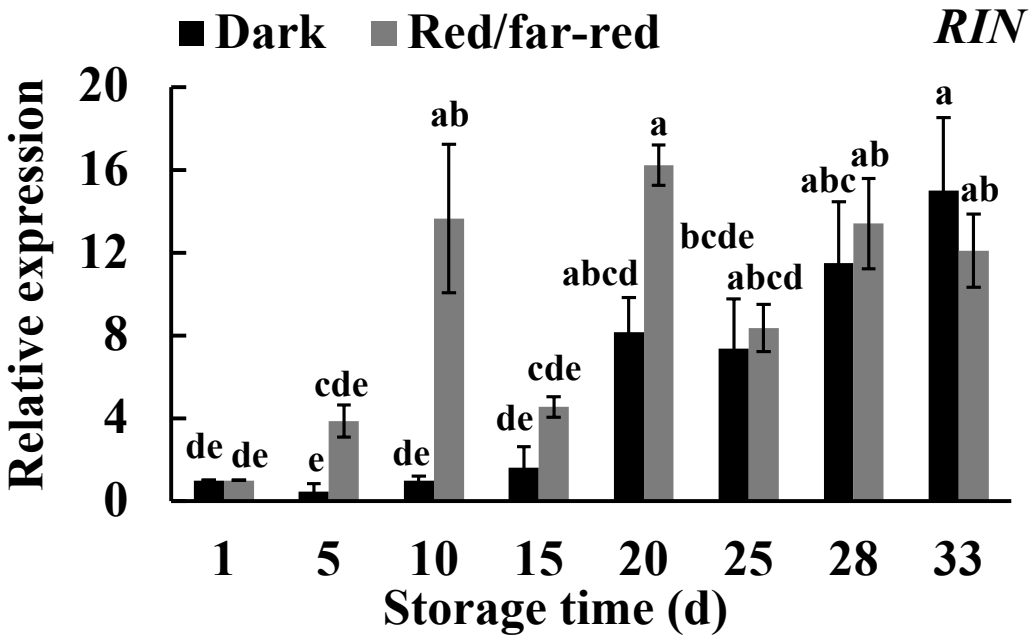


Figure 6.13 **Effects of red/far-red light on expression of ripening transcription factor gene *RIN* in cherry tomatoes during storage.** The expression level of gene was normalized against the reference gene *Actin*. Values are means ± SEs for triplicate samples. Different letters indicate that values are significantly different at $P < 0.05$.

6.4. Discussion

6.4.1. Carotenoids

In ripe red tomatoes, the major carotenoids are lycopene, ß-carotene and lutein (Schofield and Paliyath, 2005). The content of lycopene and ß-carotene increased significantly during storage, while lutein content increased during the first 10 days, but gradually decreased afterwards: this pattern is similar to that found by Ballester et al. (2010) and Xie et al. (2019). Red/far-red light induced the accumulation of lycopene and ß-carotene when compared to

darkness, and it also inhibited the induction of lutein content during storage. Schofield and Paliyath (2005) reported that red/far-red light elevated lycopene and lutein content in tomato after 14 days of storage, but ß-carotene content showed no difference in comparison to darkness. This might be caused by the ratio of red and far-red light as it has been reported that red light promoted the accumulation of carotenoid in tomato fruits, whereas far-red light reversed this effect (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010). Red and far-red light are both absorbed by phytochromes, which have the ability of detecting the ratio of red/far-red light (Xie et al., 2019). Low ratio of red/far-red light reduced PHY activity and carotenoid biosynthesis, because there will be a higher proportion of inactive Pr form (Bae and Choi, 2008; Xie et al., 2019). In this study, the red/far-red light was used together in a ratio of 0.89 (almost 1:1, far-red light was slightly higher), whereas in the study of Schofield and Paliyath (2005), red and far-red light were used separately, and the irradiation time of far-red light was three times longer than red light.

In this study, the lycopene content in fruits treated with red light increased 24.6~fold after 20 days of storage, while in control, it increased 20.7~fold. This was higher than the finding of Liu et al. (2009), who reported a 9~fold of increase of lycopene content in tomato treated with red light and a 3.5~fold in darkness after 21 days of storage. The difference in the increase of lycopene content might be related to the variety of tomatoes. Panjai et al. (2017) found the same increase of lycopene content (20.7~fold) in darkness as the result in this study, but in red light treatment, the lycopene content increased 36~fold after 20 days of storage, which was higher than the increase in red/far-red light treatment in this study. This could be explained by the evidence that red light promoted the biosynthesis of carotenoids in tomato fruits, whereas far-red light reversed this effect.

6.4.2. Transcript levels of genes involved in carotenoid synthesis

The biosynthesis of carotenoid is a complex process that is regulated by light, hormones, transcriptional and post-translational mechanisms (Alba et al., 2000a; Schofield and Paliyath, 2005). The mechanism of red/far-red light in regulating carotenoid biosynthesis has been investigated by studying the expression profiles of genes involved in carotenoid synthesis, red/far-red light absorbance and mediation, and ripening.

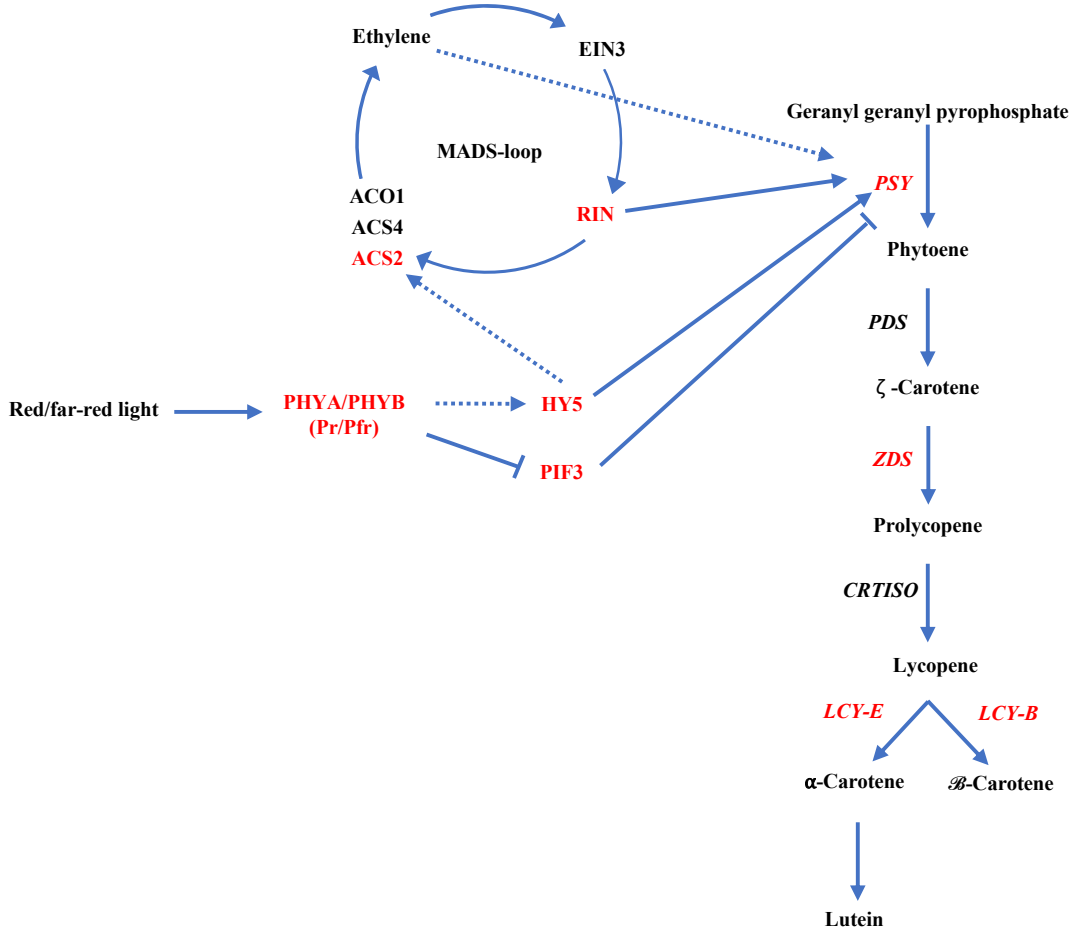


Figure 6.14 **Model for the red/far-red light mediated regulation of carotenoid biosynthesis.** Under red/far-red light (ratio 0.89) conditions, photoactivated PHYA and PHYB interacted directly with the repressive transcription factor PIF3, resulting in its degradation, and they also induced the expression of *HY5*. Elevated HY5 could either bind directly to *PSY* and promote its expression or elevate expression of *ACS2* to induce *RIN* through the MADS-loop. *RIN* could then induce the expression of *PSY* to promote

biosynthesis of lycopene. The MADS-loop was adopted from Gao et al. (2019), and the carotenoid biosynthesis pathways was adopted from Pandurangaiah et al. (2016). Arrow, positive regulation; bar, negative regulation; solid line, direct regulation; dotted line, indirect regulation.

PSY and ZDS are the rate-limiting enzymes that control the flux through the lycopene synthesis pathway (Xie et al., 2019). The expression of *PSY* and *ZDS* in red/far-red light treatment showed a similar trend during storage, registering three peaks of expression at day 10, 20 and 28, corresponding to the significant increase of lycopene content at day 10 and 28. The expression of *PSY* and *ZDS* in control also showed a similar trend during storage, which increased till day 28, and then decreased slightly, corresponding to the continuous accumulation of lycopene during storage. The expression of *PSY* and *ZDS* was higher in red/far-red light treatment than in the dark control during storage, leading to higher lycopene content in red/far-red light than in control. Xie et al. (2019) found that expression of *PSY* and *ZDS* in tomato fruits was induced by red light when compared to natural light, leading to higher lycopene content. Von Lintig et al. (1997) also reported that *PSY* transcription level was increased by continuous red light and far-red light treatment.

Lycopene can be converted to ß-carotene by the action of LCY-b or to the precursor of lutein by the action of LCY-e (Pandurangaiah et al., 2016; Xie et al., 2019). In this study, red/far-red light treatment showed higher expression of *LCY-b* gene than control at most days analysed, leading to significantly higher ß-carotene content during storage. Xie et al. (2019) found that expression of *LCY-b* was inhibited by red light in comparison to natural light. The expression of *LCY-e* in both treated and untreated fruits increased significantly to their maximum after 5 days of storage, and the expression was followed by a decline till the end of storage, corresponding to the change of lutein content during storage that it increased in

red/far-red light treatment and control at day 5 or 10, and then decreased till day 33. Xie et al. (2019) found that expression of *LCY-e* decreased during ripening.

6.4.3. Transcript levels of genes involved in light signalling pathway

The production of PSY is the first and key-limiting step in lycopene synthesis, and the transcriptional regulation of *PSY* expression is crucial to control biosynthesis of carotenoid (Maass et al., 2009; Rodríguez-Villalón et al., 2009; Toledo-Ortiz et al., 2010; Xie et al., 2019). PHYB and PHYA can perceive red and far-red light, respectively, and transduce light signals to downstream signalling intermediates, which control the expression of target genes, including *PSY* in tomato fruits (Figure 6.14) (Li et al., 2011; Schofield and Paliyath, 2005; Toledo-Ortiz et al., 2010). In this study, there were few obvious differences in *PHYA* and *PHYB* expression level found between red/far-red light treatment and control, which means that the expression levels might not be important in the phytochrome-mediated light signalling pathway that modulate carotenoid biosynthesis. Phytochromes are synthesized in their inactive Pr form (red light-absorbing from), and under red light, the Pr from can be converted to the active Pfr form (far-red light-absorbing from) (Li et al., 2011; Quail, 1997). The Pfr form can be converted back to the Pr from by much faster upon absorption of far-red light (Li et al., 2011; Quail, 1997). In this study, under the irradiation of red/far-red light, both active Pfr form and inactive Pr form will exist, and the photoactivated phytochromes can then interact with downstream transcription factors.

PIF1 and HY5 have been reported to interact with phytochromes, and they are also direct regulators of *PSY* expression which can bind to the same G-box motifs in the *PSY* promoter to regulate the biosynthesis of lycopene (Bou-Torrent et al., 2015; Toledo-Ortiz et al., 2010; Toledo-Ortiz et al., 2014; Xie et al., 2019). In this study, red/far-red light induced the expression of *HY5* during storage when compared to the control, whereas the expression of

PIF3 was not significantly affected by red/far-red light, which suggests that PHYA and PHYB mainly interact with PIF3 at the post-translational level. Altogether, we can conclude that photo-activated phytochromes induced the expression of positive regulator *HY5* to induce the expression of *PSY*, and it may also induce the decrease of negative regulator PIF3 (Figure 6.14).

6.4.4. Transcript levels of gene involved in MADS-loop

As a climacteric fruit, cherry tomato's ripening is a complex process that causes great changes in physiological and biochemical properties, including accumulation of lycopene (Dhakal and Baek, 2014a; Ioannidi et al., 2009). The hormone ethylene and the transcription factor RIN are key components in ripening and a small amount of ethylene can induce fruit ripening and the expression of downstream ripening genes, including *PSY* (Gao et al., 2019). In this study, expression of *ACS2* in red/far-red light treatment increased 19.2~fold the initial value after 10 days of storage, and 29.3~fold after 20 days, corresponding to the two peaks of *PSY* expression level at day 10 and 20 and significant increase of lycopene content at these two days in red/far-red light treatment. This suggested that the elevated expression of *ACS2* induced the transcription of *PSY* and lycopene accumulation. Yokotani et al. (2004) also reported that down-regulation of ethylene biosynthesis gene *ACS* or *ACO* led to reduced lycopene content.

RIN has also been reported to interact with the promoters of genes in the lycopene biosynthesis pathway (Martel et al., 2011; Su et al., 2015). These results agree as the transcription of *RIN* showed a similar trend to that of *PSY* in red/far-red light treated fruits during storage, registering three peaks of expression at day 10, 20 and 28, which indicates there might be direct regulation between *RIN* and *PSY*. This might support the findings of Gao et al. (2019) and Su et al. (2015), who reported that RIN could directly bind to the

promoter of ripening genes of tomato, including *PSY*. In this way, the MADS-loop is involved in the regulation of carotenoid biosynthesis. Furthermore, Ito et al. (2017) demonstrated RIN independent induction of *ACS2* and *PSY* expression in *RIN*-knockout mutation tomato, which meant that there might be indirect regulation from ethylene to *PSY*.

It has been reported that phytochrome-regulated carotenoid synthesis is related to ethylene production in red light irradiation (Alba et al., 2000a). This might mean that there is a connection between ethylene in MADS-loop and phytochrome-mediated light signalling pathway. Acting downstream of PHYA and PHYB, transcription factor HY5 directly or indirectly regulates a large number of genes (Li et al., 2011). Ge et al. (2020) reported that HY5 activated the expression of *ACS2/6/11* genes to induce the ethylene production. In this study, the expression pattern of *ACS2* in red/far-red light treatment was similar with that of *HY5* up to day 20. All of these results indicate that *ACS2* might also be a target of HY5. Overall, we can conclude that red/far-red light induced expression of *HY5* through phytochrome-mediated signalling pathway (Figure 6.14). HY5 could either directly bind to *PSY* or elevate expression of *ACS2* to induce *RIN* through MADS-loop, and *RIN* then induced the expression of *PSY* to promote biosynthesis of lycopene (Figure 6.14).

6.5.Conclusion

In this study, red/far-red light irradiation preserved postharvest quality of cherry tomatoes by inhibiting weight loss, inducing the synthesis of lycopene, ß-carotene and ascorbic acid, and inhibiting the decline of lutein and flavonoid content. Gene expression analysis showed that red/far-red light promoted the synthesis of lycopene by elevating the expression of *PSY* and *ZDS* in tomato fruits, which was modulated by phytochrome-mediated signalling pathway. Under red/far-red light irradiation, photoactivated PHYA and PHYB interacted directly with inhibitory transcription factor PIF3, resulting in the reduction of PIF3, and they also induced

the expression of *HY5*. Elevated HY5 could either directly bind to *PSY* to induce its expression or promote the expression of *ACS2* to induce *RIN* through the MADS-loop, leading to increased expression of *PSY* during the storage time (Figure 6.14). Overall results showed that red/far-red light can be used as an effective method to enhance the preservation of nutritional quality in cherry tomatoes and improve carotenoids content by regulating the expression of key ripening-related genes.

7. General discussion and future work

7.1. General discussion

The aim of this research was to investigate the effect of postharvest techniques on the nutritional quality of cherry tomatoes, and to identify strategies to preserve quality during storage. This was investigated by examining three commonly used techniques (temperature control, light irradiation and MAP) and measuring a number of parameters of postharvest quality, including colour change, weight loss, and the content of nutritional components (ascorbic acid, lycopene, β-carotene, lutein, total phenolics and flavonoids).

7.1.1. The influence of temperature on fruit quality

Temperature control is the simplest and most important procedure for the preservation of produce (Brasil and Siddiqui, 2018). In order to provide some initial evidence on the effect of temperature on cherry tomatoes, three temperatures 5, 15 and 20°C, representing chilling injury sensitive, storage suitable and ripening suitable temperature, were examined. Of these temperatures, 15°C was demonstrated to be the most suitable temperature to extended shelf-life of cherry tomatoes.

Fruits stored at 15°C slowed the ripening process compared to 20°C, while fruits stored at 5°C failed to develop beyond the pink stage and showed symptoms of chilling injuries; this outcome is consistent with the finding of Gomez et al. (2009) and Cantwell (2000). Although Cantwell (2000) reported that tomato fruits stored at 15-20°C had longer shelf-life than fruits stored at higher temperatures, we believe that this is the first time that the difference between 15 and 20°C has been examined.

Fruits stored at 15°C had significantly lower weight loss than at 20°C during storage, this is in agreement with Javanmardi and Kubota (2006), Mangaraj et al. (2019) and Choi et al. (2015a), who reported that weight loss of fruits stored at temperature above 20°C was significantly higher than that at lower temperature (such as 12, 10 and 4°C). This is because fruits stored at higher temperatures (above 20°C) had higher respiration and transpiration rates (Guo et al., 2019; Murmu and Mishra, 2018). Interestingly, this study found that weight loss of fruits stored at 15°C was significantly lower than that at 5°C during storage; one explanation might be that cherry tomatoes stored at 5°C developed chilling injuries that caused physiological disorders (Mangaraj et al., 2019; Park et al., 2018b).

Of the three temperatures examined, 15°C was the most suitable to preserve nutritional quality of cherry tomatoes. For instance, fruits stored at 15°C for 28 days had significantly the highest lycopene and β-carotene content, although no significant difference was found in the content of lutein and flavonoid, it was the highest in fruits stored at 15°C. Whereas temperature of 5°C inhibited the production of lycopene and β-carotene.

The content of lycopene and β-carotene in fruits stored at 20°C was the highest till day 20, then decreased from day 25, suggesting that higher temperatures may accelerate the conversion these components to others, as according to Hirschberg (2001), lycopene can be converted into carotenes, and β-carotene can be converted into zeaxanthin by the action of relative enzymes.

Overall, we found that 15°C could delay the ripening, inhibit weight loss of fruits, and preserve the nutritional quality in terms of the content of lycopene, ß-carotene, lutein and flavonoids in cherry tomatoes till 28 days, and it is more suitable for long-term storage in

comparison to 20°C. Fruits stored at 20°C had the fastest ripening process, and they are more suitable to be consumed before day 20 after harvested. We are not aware of any previous publication highlighted this finding, so our study added new finding in the research of storage temperature's effects on cherry tomatoes.

7.1.2. The influence of light on fruit quality

Light irradiation has been shown as another effective postharvest treatment (Hasan et al., 2017) so postharvest treatment of fruits with red/far-red and blue light irradiation was also investigated. Most research to date has focused on the application of red or blue light on round-type tomatoes or other vegetables (Ma et al., 2014; Shi et al., 2014; Xu et al., 2014), and there is limited research about the application of red/far-red light or blue light on cherry tomatoes. The experiment was designed to address that data gap.

The results showed that fruits treated with red/far-red light developed deeper red colour and had lower weight loss compared to darkness control, whereas blue light had little effect on the development, and blue light resulted in greater weight loss during 33 days of storage. Dhakal and Baek (2014b) reported similar result that blue light could delay colour changing from green to red in cherry tomatoes, while Hasperué et al. (2016) reported that Brussels sprout exposed to blue light had higher weight loss in comparison with darkness. The reason that blue light irradiation induces weight loss in leaf vegetables is that it motivates stomatal conductance and transpiration, which results in increased moisture loss during storage (Hasan et al., 2017). Although cherry tomato fruits do not have stomata, they lose water by transpiration mainly via trichome-associated transcuticular polar pores, which might be the way that blue light work (Fich et al., 2020).

Red/far-red light irradiation increased ascorbic acid content when compared with darkness, whereas blue light showed little effect. Red/far-red light irradiation promoted the accumulation of lycopene and β-carotene content significantly when compared to darkness, and inhibited the reduction of lutein content during storage, whereas long-term blue light showed little effect on lycopene content. Ma et al. (2012) reported that red light could increase carotenoid content, while blue light had no significant influence on it in citrus fruit. In this study, cherry tomatoes stored in long-term red/far-red light treatment had the highest content of total phenolics at the end of storage. Similar results were reported by Panjai et al. (2017) who found that tomatoes treated with red light had higher total phenolic content than that in darkness.

Overall, red/far-red light could maintain postharvest quality of cherry tomatoes by inhibiting weight loss, inducing colour changing from green to red, promoting accumulation of lycopene, β-carotene, and total phenolics content, and inhibiting reduction of lutein content of cherry tomatoes during transportation and storage.

Besides light quality, light quantity - irradiation duration, is also an important factor that might affect plant quality (Zheng et al., 2019). To explore the influence of the light duration on cherry tomatoes, we used three durations 5, 15 and 33 days of red/far-red and blue light to represent short-, middle, and long-term light irradiation.

After 33 days of storage, no significant differences were shown in all three red/far-red light treatments in all nutritional components – ascorbic acid, lycopene, β-carotene, lutein, total phenolics and flavonoids. The results suggest that 5 days' irradiation is long enough to preserve the nutritional quality of cherry tomatoes, in the meanwhile, it shortens light

irradiation time to save energy. Comparable results were found in the blue light treatment that fruits treated for 5 days might be a better choice.

7.1.3. The influence of the combination of MAP and red/far-red light on fruit quality

Red/far-red light has been demonstrated to extend shelf life and preserve nutritional quality of cherry tomatoes during storage, but we extended the work to examine the influence of both red/far-red light and MAP on fruit quality. Currently, most cherry tomatoes are packed using MAP and exposed to fluorescent lamps (400-700 nm in wavelength), so adding red/far-red light may reveal different or enhanced outcomes.

Results showed that passive MAP combined with red/far-red light could extend shelf-life of cherry tomatoes compared to fluorescent white light control. The combination delayed the increase of respiration and colour changing to delay ripening, and it inhibited weight loss compared with a white light control. MAP, on its own, also inhibited weight loss, but it showed fast ripening process. Less weight loss in MAP was due to the low water vapor transmission rates which when combined with the high respiration activity of cherry tomatoes inside of the packaging, results in very high relative humidity within the packing (Antmann et al., 2008; Ye et al., 2012). A similar result was also found by Elwan et al. (2015), Fagundes et al. (2015) and Ye et al. (2012).

Passive MAP had higher level of ascorbic acid, lycopene, ß-carotene, lutein, total phenolics and flavonoids at most days of storage compared with control, whereas red/far-red light showed lower content of these nutritional components. Ye et al. (2012) reported similar results showing that MAP inhibited the decrease of ascorbic acid content in mushrooms when compared with control. Fagundes et al. (2015) also found that MAP induced the

accumulation of lycopene in cherry tomatoes compared to control. Murmu and Mishra (2018) and Domínguez et al. (2016) found that total phenolics was induced by MAP at the most days of storage.

The combination of passive MAP and red/far-red light effectively increased the content of lycopene and ß-carotene at most days of storage, indicating the dominant contribution of MAP on their accumulation in the fruits with combined treatment. However, for other components, induction caused by MAP was blocked by R/FR irradiation.

7.1.4. The molecular mechanism of red/far-red light in the regulation of carotenoid biosynthesis

Early research in this study showed that red/far-red light significantly enhanced the content of carotenoid in comparison to darkness so later work examined the role and mechanism of red/far-red light in the regulation of carotenoid biosynthesis.

The expression of the two lycopene biosynthesis genes *PSY* and *ZDS* that together primarily control the flux through the carotenoid biosynthesis pathway, showed a similar trend during storage, and was induced by red/far-red light treatment, leading to higher lycopene content in red/far-red light than in control. This is consistent with Xie et al. (2019), who found that expression of *PSY* and *ZDS* in tomato fruits was induced by red light when compared to natural light, leading to higher lycopene content. Red/far-red light induced the expression of *LCY-b* gene at most observations, leading to significant higher ß-carotene content during storage.

Red/far-red light photoreceptor genes *PHYA* and *PHYB* expression level showed no significant difference between red/far-red light treatment and control, suggesting that the

transcription levels of them are less important in the phytochrome-mediated light signalling pathway that modulate carotenoid biosynthesis. Instead, their active Pfr/inactive Pr form conversion under red/far-red light will decide the interaction with downstream transcription factors (Li et al., 2011; Quail, 1997).

Red/far-red light induced the expression of *HY5* during storage, whereas the expression of *PIF3* was not significantly affected, which suggests that PHYA and PHYB mainly interact with PIF3 at the post-translational level. Altogether, we can conclude that photo-activated phytochromes induced the expression of *HY5* and the degradation of PIF3, and the increase of positive regulator HY5 and the decrease of negative regulator PIF3 induced the expression of *PSY*.

The expression of ethylene biosynthesis *ACS2* in red/far-red light treatment increased significantly at day 10 and 20, corresponding to the two peaks of *PSY* expression level and significant increase of lycopene content at these two days in red/far-red light treatment. This suggested that the elevated expression of *ACS2* induced the transcription of *PSY* and lycopene accumulation. The transcription of *RIN* showed a similar trend as that of *PSY* in red/far-red light treated fruits during storage, registering three peaks of expression at day 10, 20 and 28, which indicates the possibility that there is a direct regulation between *RIN* and *PSY*. This could be supported by the findings of Gao et al. (2019) and Su et al. (2015), who reported that RIN could directly bind to the promoter of ripening genes of tomato, including *PSY*. Ge et al. (2020) reported that HY5 activated the expression of *ACS2/6/11* to induce the ethylene production under UV-B irradiation. In this study, the expression pattern of *ACS2* in red/far-red light treatment was similar with that of *HY5* during storage, which indicates that *ACS2* might be the target of HY5.

Overall, we can conclude that red/far-red light induced expression of *HY5* through phytochrome-mediated signalling pathway. HY5 could either directly bind to *PSY* or elevate expression of *ACS2* to induce *RIN* through MADS-loop, and *RIN* then induced the expression of *PSY* to promote biosynthesis of lycopene.

7.1.5. Overall discussion

In the storage of cherry tomatoes, temperature control is the simplest approach. When harvested at mature green stage, fruits stored at 20°C have faster ripening process and shorter shelf life than those at 15°C, and they are more suitable to be consumed before day 20 to obtain a high level of nutrition. While 15°C can delay the ripening and preserve the nutritional quality till 28 days, and it is more suitable for long-term storage in comparison to 20°C.

The application of red/far-red light at 15°C can further extend the shelf life till 33 days. Compared with darkness control, red/far-red light can maintain postharvest quality of cherry tomatoes by inhibiting weight loss and preserving nutritional quality of cherry tomatoes during transportation and storage. MAP is a good method to preserve nutritional quality of cherry tomato, and the combination with red/far-red light might have the potential to replace the white light.

Through the analysis of relative gene expression levels, we identified the signalling pathway that promotes lycopene accumulation, which is by inducing *HY5* through phytochrome-mediated signalling pathway to either directly bind to *PSY* or promote the expression of *ACS2* to induce *RIN* through MADS-loop, and *RIN* then induces the expression of *PSY* to promote biosynthesis of lycopene.

7.2.Future works

This research provided strategies for the storage of cherry tomatoes from temperature, light irradiation and the combination with MAP. And it also provided a hypothetical model of red/far-red light in the regulation of carotenoid biosynthesis.

However, all postharvest treatments were applied to one variety of cherry tomato (cv. Piccolo) in this study, and some nutritional components might be cultivar dependent (Nájera et al., 2018). Further studies need to be carried out using more varieties of tomato to explore if the results in this study can be applied to other types of tomato. For example, the effect of temperatures on weight loss, colour change and the content of nutritional components in 'Dometica', 'Juanita' and even other types of tomato, such as plum tomatoes, classic round tomatoes need to be carried out.

We used three temperature (5, 15, 20°C) treatments in this study to represent chilling injury sensitive, storage suitable and ripening suitable temperature, respectively. Further studies need to be carried out to narrow down the range between 15-20°C, to explore an optimum temperature the storage of cherry tomatoes to preserve the nutritional quality.

When explored the effects of red/far-red and blue light on nutritional quality of cherry tomatoes, we used darkness as the negative control. It would be useful to add a positive control-white light, to find out if red/far-red light is a better approach to preserve quality of cherry tomato when compared to white light.

In the pre-experiment in Chapter 5, more parameters, such as firmness and appearance should have been measured for NPP, MPP15, MPP30 and HPP, to find out the best one to be used as

MAP treatment for formal experiment. And the light quality and quantity used for this experiment should be measured insides of the packages as well, to avoid the potential effects of film package. And also, using light lamb tubes instead of light filters would be a better choice to make sure the same light intensity for different lights.

The mechanism of red/far-red light in the regulation of carotenoid biosynthesis was based on the analysis of gene expression levels, further studies can be done by using knock-out or over-expression mutants of these genes (such as *ACS2*, *PIF3* and *RIN*) to apply more evidence for the signalling pathway model. In addition, the red/far-red light ratio is an important factor that affects the transition of inactive Pr and active Pfr form of phytochromes. In this study, we only used one ratio (0.89) of red/far-red light treatment. Further studies need to be carried out with lower or higher ratio of red/far-red light to explore the effect on carotenoids in cherry tomatoes.

Finally, it would be very useful to carry out full transcriptomics experiments using RNA sequencing, to find out the differences of gene expression pattern under red/far-red light, darkness and even white light, relative to carotenoid biosynthesis, and to explore the light signalling pathway.

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