ORIGINAL ARTICLE

Changes in carotenoid and chlorophyll content of black tomatoes (*Lycopersicone sculentum* L.) during storage at various temperatures

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Abstract   Black tomatoes have a unique color and higher lycopene content than typical red tomatoes. Here, black tomatoes were investigated how maturation stage and storage temperature affected carotenoid and chlorophyll accumulation. Immature fruits were firmer than mature fruits, but failed to develop their distinctive color and contained less lycopene when stored at 8 °C. Hunter a* values of black tomatoes increased with storage temperature and duration; storage of immature fruits at high temperature favored lycopene accumulation. Chlorophyll levels of black tomatoes declined during storage, but differences between mature and immature tomatoes stored at 12 °C were minimal. β-Carotene levels of black tomatoes increased during early storage, but rapidly declined beginning 13 d post-harvest. The highest lycopene and chlorophyll levels were observed in mature black tomatoes stored at 12 °C for 13 d; these conditions also yielded the best quality fruit. Thus, the unique pigmentation properties of black tomatoes can be precisely controlled by standardizing storage conditions.

1. Introduction

Tomatoes are consumed globally as healthy fresh fruits and processed food. They play a key role in the human diet because of the high functionality of many of their constituent compounds. Tomato ranked first as the major source of lycopene followed by β-carotene and vitamins C and E (García-Closas et al., 2004). Carotenoids, which are plant pigments, act as antioxidants that prevent oxidative modification of human plasma low-density lipoprotein (LDL) (Oshima et al., 1996). Lycopene is the most effective antioxidant for radical scavenging among the carotenoids (Mortensen and Skibsted, 1997). Lycopene and β-carotene are also major contributors to tomato fruit pigmentation, as they are responsible for the deep red and orange colors of the pericarp tissue, respectively (Tijsken and Evelo, 1994). The change in fruit color during tomato ripening is due to a transition from chloroplasts to chromoplasts, which is brought about by chlorophyll degradation and carotenoid

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synthesis (Liu et al., 2009). Lois et al. (2000) reported two enzymes are coordinated especially in controlling carotenoid synthesis during the ripening of tomato fruit. The D-xylulose 5-phosphate synthase (DXS) which is responsible for chlorophyll synthesis in green tissue and carotenoid synthesis during early fruit ripening and phytoene synthase (PSY1), a fruit specific isoform, that regulates carotenogenes is showing an increase in gene expression level during fruit ripening.

Carotenoid synthesis, particularly lycopene accumulation, correlates with tomato fruit color (Cox et al., 2003) and is significantly affected by storage temperature. Chlorophyll levels are sensitive to low temperature and are thus used as a chilling injury index (Hershkovitz et al., 2005). Fruits stored at 8 °C fail to develop a robust red color (Gómez et al., 2009; Javanmardi and Kubota, 2006). By contrast, storage at 12 °C or 17 °C for up to 21 d engenders an optimal color in cherry tomatoes (Fuchs et al., 1995). The optimal ripening condition for the typical standard-size red tomatoes is slightly higher, and lies between 18 °C and 21 °C. Temperature below 5 °C and 10 °C for longer than 7 and 14 d, respectively, prevents ripening and full color development (Suslow and Cantwell, 2013). Changes in pigmentation during postharvest storage may affect the quality of the tomato fruit because its skin color is associated with ripening stage and a marketable flavor. However, the levels of lycopene, phenolic compounds (flavonoids and hydroxycinnamic acids), and antioxidant activity are significantly influenced in a variety- and maturity stage-dependent manner (Martínez-Valverde et al., 2002). Generally, the recommended harvest time for typical red tomatoes is at the mature green, breaker or pink stages, as this favors a long shelf life and is associated with optimal fruit firmness. However, immature fruit does not fully develop its flavor during ripening (Wills and Ku, 2002). In contrast, harvesting at the colored stage in which lycopene content is high is associated with a flavorful fruit, albeit with a shorter shelf life.

The black tomatoes are reported to have a blackish red skin with higher lycopene content (185 mgkg^{-1}) compared to both typical red tomatoes (Martínez-Valverde et al., 2002; Raffo et al., 2006; Seo et al., 2013). Unlike typical red tomatoes, the black tomatoes retain chlorophyll along with lycopene synthesis and develop a noticeable blackish-red skin (Ekelund and Jönsson, 2011). During ripening of the 'Kumoto' black tomatoes, reduced chlorophyll degradation results from a green-fresh (gf) mutation (Hu et al., 2011). However, the mechanisms of carotenoid synthesis and chlorophyll degradation during ripening under low temperature storage conditions remain unclear. Furthermore, the contribution of these bio-molecules to fruit quality and the storability of the black tomatoes are still unclear. In this study, therefore, black tomatoes were investigated the effects of storage temperature and storage time on the levels of carotenoid and chlorophyll accumulation in fruit harvested at different stages of maturity. Our results provide clear guidelines for the proper storage conditions required to provide high-quality black tomatoes for consumers.

2. Materials and methods

2.1. Plant material and treatment condition

Black tomatoes (Lycopersicon esculentum cv. 'Hei') were planted in a plastic house in Jeollanam-do, Damyang, South Korea. 'Hei' is a cultivar of red with black flesh; originated from the cross of two inbred lines, TUKI separated from 'Kame' and TLB separated from tomato collected in Europe. The fruit shape is round and fruit color is blackish red. Immature (40–50% maturity; dark green color) and mature (70% maturity; conventional harvest stage of blackish red color) fruits were harvested on July 10th, 2014 and stored separately at 8 °C, 12 °C or 20 °C for 20 d.

2.2. Quality evaluation

Weight loss was repeatedly measured in three replicates (tray). Each replicate contained 15 fruits. Skin color from two opposite of each fruit was measured using a color difference meter (Minolta CR-400 model, Osaka, Japan) and reported according to Hunter's scale; L*, a* and b* values and a ratio of a*/b*. The firmness of each peeled fruit sample was determined using a texture analyzer (TA Plus Lloyd Instruments Ltd., Fareham, Hamp shire, UK). Fifty fruits per replicate were penetrated at a speed of 2 mm·s^{-1} with a 5-mm diameter plunger head. Soluble solids content (SSC) was determined using a digital refractometer (PAL-1, Atago Co. Ltd., Japan) with juice samples. Titratable acidity (TA) was determined by titrating 5 ml of juice with 0.1 N NaOH using an auto-pH titrator (Titroline easy; SCHOTT instruments GmbH, Mainz, Germany) and expressed as citric acid content.

2.3. Respiration and ethylene production

Respiration and ethylene production were analyzed using a gas chromatograph (Bruker 450-GC model, USA) equipped with aflame ionization detector (FID) and thermal conductivity detector (TCD). Gas samples (1 mL) were withdrawn using a syringe from 2 h sealed 1Lcontainers containing 3fruits from each treatment. Respiration rate was determined based on carbon dioxide production level using a TCD. Ethylene production was measured using a FID with an active alumina column. An external standard of ethylene (10 μL L^{-1}) was used for calibration. The injection and column temperatures were 110 °C and 70 °C, respectively. Both TCD and FID detectors used for CO_2 and ethylene productions were set at 150 °C and 250 °C, respectively. High-grade helium was used as the carrier gas for ethylene and carbon dioxide detection at a flow rate of 0.5 mL s^{-1}.

2.4. Chlorophyll analysis

The chlorophyll content was analyzed by modifying the AOAC method (AOAC, 1965; Kozukue and Friedman, 2003). Frozen tomato fruit pericarp sections (10 g) were homogenized by grinding with 10 mL of 80% acetone containing 0.1 g $MgCO_3$ in a glass mortar. The mixture was homogenized in a 50 mL Falcon tube at 28,000g for 1 min, and then centrifuged at 18,100g for 10 min at 1 °C. The pellet was repeatedly extracted and centrifuged three times with 10 mL of 80% acetone and the supernatants were then combined. This solution was then analyzed using a UV spectrophotometer (EPOCH2 microplate reader, BioTek Instruments Inc., USA) at 665 nm and 642.5 nm for chlorophyll a and b, respectively.

2.5. Carotenoid analysis

Lycopene and β-carotene extraction and analyses were performed using modifications of previous methods (Fraser et al., 2000; Howe and Tanumihardjo, 2006). Freeze-dried powder of tomato fruit pericarp (0.4 g) was shaken with 5 mL of ethanol in a 50 mL Falcon tube for 5 min at 75 °C in a warm water bath. KOH (1.5 mL of an 80% solution) was added and the mixture was shaken again for another 10 min in the same warm water bath. The reaction was then stopped by shaking on ice for 5 min. Ultra-pure water and hexane (2 mL each) were added and vortexed. The solution was centrifuged at 1000g for 3 min at 4 °C. The hexane layer was collected and transferred into a flask. This process of adding ultra-pure water and hexane mixture followed by centrifugation was repeated twice (for a total of three replications). The combined hexane layer supernatants were concentrated in a pressure concentrator. The concentrated solution was adjusted to 1 mL (v/v) with a mixture of methanol:dichloromethane (50:50), then filtrated with a 0.20 μm PTFE hydrophilic syringe filter with a diameter of 13 mm and kept in a vial for UPLC analysis.

Lycopene and β-carotene was quantified using the ACQUITY UPLC (Waters, USA) instrument equipped with an ACQUITY UPLC BEH C18 column (130Å, 1.7 μm, 2.1 mm × 100 mm; Waters, USA). The oven temperature was 30 °C. The mobile phases were methanol: water (75:25 v/v; solvent A) and ethyl acetate (solvent B) at a flow rate of 0.2 mLmin^{-1}. Analytes were eluted using a gradient: initially 100% to 0% solvent A over 6 min, followed by 0% solvent A for a further 4 min, then 0% to 100% solvent B over 2 min and reduced to 0% solvent B over 3 min. Detection was performed at 475 nm for lycopene and 450 nm for β-carotene. The analyzed compounds were identified by comparing their elution times with those of authentic standards. The lycopene and β-carotene peaks were displayed on the HPLC chromatogram (Fig. 1).

2.6. Statistical analysis

Values are the mean, plus or minus standard error. Samples were subjected to analysis of variance (ANOVA) and significant differences were determined using Duncan's multiple range test (DMRT) in the SAS 9.2 statistical software package (SAS Institute, Cary, USA).

3. Results and discussion

3.1. Quality changes of black tomato fruit harvested at different maturity stages and storage temperatures

The quality of black tomato fruits during the early period of storage at different temperatures was affected by the fruit maturity stage at harvest. Immature fruits at harvest were 4-fold firmer and had double the TA content of mature fruits; however, the SSC was similar in both cases. Immature black tomato fruits ripened normally during storage (Table 1), similar to observations from studies of the typical red tomato (Liu et al., 2009). In black tomatoes, the firmness of immature fruits continuously decreased during storage from 10.45N to 5.10N, which is indicative of a normal ripening process, although the values were slightly higher than those observed with mature fruit at the same time (4.50N) when stored at 8 °C and 12 °C. Weight loss of both immature and mature black tomato fruits gradually increased upon storage temperature, from 2.25% to 5.43% for an immature fruit, and from 1.91% to 7.18% for a mature fruit at 20 d, respectively (Table 1). However, there was no significant difference in weight loss between immature and mature fruits when stored at 8 °C and 12 °C. The change in SSC during low-temperature storage was 4.32–5.28% and was not affected by maturation stage of the black tomatoes. SSC is reportedly even higher after removal from 12 °C storage and subsequent storage at 20 °C for 5 d (Lurie and Klein, 1992). Even though maturity at harvest affects fruit sweetness in many typical red tomatoes, our results indicate that the SSC of the black tomatoes harvested at both stages was not significantly different over 9–20 d of storage at 12 °C. In black tomatoes, immature fruit had twice the TA content of mature fruit, indicating higher acidity than mature fruits (Table 2). In contrast to the tradition of harvesting at the mature green stage for many other cultivars (Lurie and Klein, 1992; Thomson et al., 2000; Kozukue and Friedman, 2003), black tomatoes are best harvested at 70% maturity or the blackish-red stage to provide better fruit quality and storability.
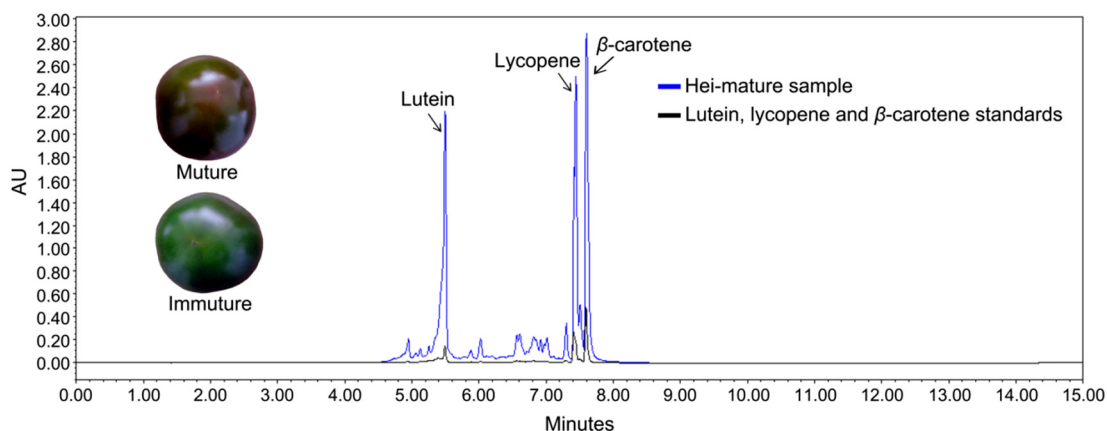

Figure 1 HPLC chromatogram of black tomatoes lutein, lycopene, and β-carotene.

Table 1 Changes in firmness and weight loss of immature (dark green color) and mature (conventional harvest stage of blackish red color) black tomatoes during storage at various temperatures for 20 d.

Maturity Stages	Storage Temp.	Storage period (days)						
		0	2	6	9	13	16	20
Firmness (N)								
Immature	8 °C	10.45 ± 2.78A[z]	7.63 ± 2.51aA	4.29 ± 1.86cAB	7.53 ± 2.67aA	6.47 ± 2.45abA	4.91 ± 2.14bcA	5.43 ± 2.36bcA
	12 °C		8.03 ± 2.07aA	5.41 ± 1.81bcA	6.49 ± 2.38abAB	4.75 ± 1.30bcBC	4.42 ± 1.68cA	5.10 ± 2.05bcA
	20 °C		5.10 ± 1.24aB	3.43 ± 1.22bBC	3.06 ± 0.60bcD	3.11 ± 0.66bcD	2.37 ± 1.14cC	2.25 ± 0.89cB
Mature	8 °C	3.89 ± 0.78B	4.70 ± 1.37abB	3.53 ± 1.53bBC	4.53 ± 1.40abCD	4.42 ± 0.81abBC	4.28 ± 1.21abAB	4.85 ± 1.67aA
	12 °C		4.71 ± 1.17abB	3.97 ± 1.50abBC	5.24 ± 1.37aBC	5.22 ± 1.82aAB	3.74 ± 1.38bABC	4.50 ± 1.12abA
	20 °C		4.89 ± 1.18aB	2.82 ± 1.14bC	3.16 ± 0.73bD	3.54 ± 0.8bCD	2.84 ± 1.40bBC	2.80 ± 0.78bB
Weight loss (%)								
Immature	8 °C	0.00	0.18 ± 0.09cA	0.36 ± 0.48cB	0.78 ± 0.53bcC	1.19 ± 0.38bC	1.53 ± 0.47abC	2.20 ± 0.40aB
	12 °C		0.10 ± 0.05cA	0.77 ± 1.25bcB	1.57 ± 1.20abcBC	2.22 ± 1.20abcBC	2.83 ± 1.14aC	3.54 ± 1.03aB
	20 °C		0.14 ± 0.03fA	2.32 ± 0.90eA	3.97 ± 0.69dA	5.91 ± 0.39cA	7.31 ± 0.22bA	8.93 ± 0.08aA
Mature	8 °C	0.00	0.20 ± 0.06cA	0.45 ± 0.50bcB	0.76 ± 0.47abcC	1.18 ± 0.64abcC	1.45 ± 0.72abcC	1.91 ± 0.89aB
	12 °C		0.16 ± 0.06dA	0.83 ± 0.85cdB	1.43 ± 0.78bcdBC	2.12 ± 0.74abcBC	2.66 ± 0.78abcBC	3.32 ± 0.84aB
	20 °C		0.10 ± 0.05dA	0.53 ± 0.38cdB	1.84 ± 0.66bcdB	3.55 ± 1.30bcB	5.08 ± 2.16abB	7.18 ± 3.40aA

[z] Means with the same uppercase letter in the column or same lowercase letter in the row were not significantly different at P < 0.05 with Duncan's multiple range test.

In black tomatoes, mature fruit could be stored at 8 °C and 12 °C for up to 20 d without a loss of fruit firmness, and in the absence of changes to SSC and TA (Table 2). In contrast, mature fruit stored at 20 °C lost up to 7.18% water, which resulted in fruit softening, as fruit firmness decreased and reached its lowest levels by 20 d post-storage (Table 1). As indicated by CO_2 production of black tomatoes, the respiration of mature fruit stored at 20 °C was higher than that of fruit stored at 12 °C and 8 °C, for all time points studied. Respiration of immature fruits was higher than that of mature fruit when compared at the same storage temperature. Together, these results indicate that immature black tomato fruit still contained the secondary metabolism required for energy.

Ethylene production in black tomatoes depends on storage temperature. Mature fruit stored at 20 °C reached a climacteric peak as early as 6 d of storage (3.6 µg kg^{-1} s^{-1}), and the values then declined slightly but remained high (2.3 µg kg^{-1} s^{-1}) even after 16 d of storage (Fig. 2). Mature fruit stored at 8 °C and 12 °C produced the highest amount of ethylene during the first 2 d of storage, i.e., 2.8–3.1 µg kg^{-1} s^{-1}, and the amount then gradually decreased before reaching the lowest point after 16 d of storage (0.6–0.9 µg kg^{-1} s^{-1}). The climacteric peak of ethylene evolution of this black tomato during storage at 12 °C was twofold lower than that reported for the 'Rehovot 121' tomato (Lurie and Klein, 1992). However, maximum ethylene production of black tomatoes occurred in immature fruit following 9 d of storage at 20 °C. Because ripening of green tomatoes is accelerated by exposure to ethylene (Lurie and Klein, 1992), these data indicate that 9 d is the time point at which ripening begins. In contrast to storage at 20 °C, immature fruit stored at 8 °C or 12 °C produced less ethylene during storage and was more delayed in ripening (Fig. 2), which indicates that immature black tomatoes require storage at 20 °C for optimal ripening, and also that delayed ripening maintains fruit firmness. The higher respiration and ethylene production of black tomatoes also affects weight loss, which is supported by the observation that maximum weight loss occurred at high storage temperature, and when respiration was high in both mature and immature black tomatoes. With regard to fruit quality of black tomatoes, storage of mature fruit at 8 °C and 12 °C appears to favor the emergence of the most desirable properties .The weight loss of black tomatoes stored at 8 °C was lower than that of fruits stored at 12 °C; however, this difference did not have statistical significance, and analysis of the SSC/TA ratio indicated that better flavor occurred with storage at 12 °C for 13 d.

3.2. Skin color and pigment development during storage

In black tomatoes, immature fruits stored at 8 °C failed to ripen even after 20 d of storage. The red skin color of immature fruits, as estimated by Hunter's a* value, developed slowly during storage at 8 °C, whereas at 12 °C and 20 °C the color significantly increased, reaching a peak at 5.9 and 6.8 after 20 d storage, respectively. These findings are similar to those observed with mature fruits before storage. Together, these results indicate that immature fruits stored at 12 °C and 20 °C are unable to evolve a blackish red skin color of black tomatoes. By contrast, the skin color of mature fruits developed better than that of immature fruits during storage at all

Table 2 Changes in solid sugar content (SSC) and titratable acid (TA) of immature (dark green color) and mature (conventional harvest stage of blackish red color) black tomatoes during storage at various temperatures for 20 d.

Maturity Stages	Storage Temp.	Storage period (days)						
		0	2	6	9	13	16	20
SSC (%)								
Immature	8 °C		4.72abA	4.90aAB	4.90aA	4.76abA	4.76abA	4.38bAB
	12 °C	5.28A[z]	5.02aA	5.02aA	4.88aA	4.74aA	4.70aA	4.70aA
	20 °C		4.86aA	4.94aAB	4.88aA	4.50aAB	4.56aA	4.38bAB
Mature	8 °C		4.58aA	4.46aBC	4.24aBC	4.24aAB	4.30aA	4.58aAB
	12 °C	4.94A	4.68aA	4.32aC	4.66aAB	4.56aAB	4.52aA	4.74aA
	20 °C		4.74aA	4.52abBC	4.12cC	4.16bcB	4.38abcA	4.22bcB
TA								
Immature	8 °C		0.69aA	0.54bA	0.46bB	0.43bB	0.41bA	0.43bA
	12 °C	0.58A	0.56abAB	0.51abcA	0.69aA	0.54abA	0.41bcA	0.33cB
	20 °C		0.47aBC	0.39bB	0.33bcBC	0.28cdC	0.24 dB	0.25cdC
Mature	8 °C		0.27aD	0.24aC	0.16bD	0.23aC	0.21abB	0.23aC
	12 °C	0.25B	0.24aD	0.21aC	0.29aCD	0.26aC	0.23aB 0.22bB	0.28aC
	20 °C		0.34aCD	0.21bC	0.20bCD	0.23bC	0.22bB	0.22bC

[z] Means with the same uppercase letter in the column or same lowercase letter in the row were not significantly different at P < 0.05 with Duncan's multiple range test.

three temperatures. In contrast to their immature counterparts, the skin color of mature fruits stored at 12 °C and 20 °C was not significantly different (Table 3).

Hunter's b* value of immature fruits stored under all three temperature conditions decreased continuously during the first 9 d of storage, then remained constant. In contrast, the b* values of mature fruits stored at 8 °C and 12 °C slightly decreased during the first 9 d of storage, then slightly increased again. The b* values of fruits stored at 20 °C remained constant at all times (Table 3).

The Hunter's a*/b* ratio (indicative of the red ripeness stage) of both immature and mature black tomatoes stored at all three temperatures continuously increased and reached a peak at 20 d of storage. This ratio indicated that immature fruit stored at 8 °C for 20 d (0.03) did not ripen normally. However, those stored at 12 °C or 20 °C for 20 d (0.38 and 0.46, respectively) developed a red color similar to those observed in mature fruits at harvest (0.40) and after storage at 8 °C for 20 d (0.46), respectively (Table 3). In contrast to typical red tomatoes (Brandt et al., 2006), which are associated with a*/b* ratios between 1.0 and 1.18, the highest a*/b* ratios of our mature black tomato fruits stored at 12°C or 20 °C for 20 d were between 0.66 and 0.68, respectively. These ratios were significantly higher than with mature fruit at 8 °C on the same day (0.46), probably because of incomplete chlorophyll degradation during ripening of black tomato fruits (Table 5).

3.3. Changes in the carotenoid and chlorophyll content of black tomatoes during storage

The levels of functional compounds including carotenoids (Table 4) and chlorophyll (Table 5) both at harvest and during storage are considered important indicators of the fruit quality and storability of black tomatoes. Because temperature also affects the levels of these compounds, a quantitative analysis of each compound was performed during storage of the black tomatoes.

3.4. Lycopene and β-carotene

Our HPLC analysis indicated that mature fruit at harvest contained double the amount of lycopene compared to immature fruits. This is consistent with reports that tomato lycopene content depends on maturity and ripeness at harvest (Kozukue and Friedman, 2003; Lurie and Klein, 1992). The lycopene content of both immature (50.5 mg kg^{-1}d.w.) and mature black tomatoes (105.5 mg kg^{-1}d.w.) at harvest was 2- and 4-fold higher than that found in mature green 'Red Ruby' tomatoes (Liu et al., 2009). In typical red tomatoes, lycopene accumulation is inhibited following storage at 12 °C for 7 d followed by 5 °C for another 7 d (Javanmardi and Kubota, 2006); it can also be accelerated between 4 and 21 d of storage (Alba et al., 2000; Liu et al., 2009). By contrast, the lycopene content of both immature and mature black tomatoes our current study increased suddenly after initiation of storage in all conditions, and reached a peak at 13 d post-storage before declining. The lycopene content of both immature and mature black tomatoes stored at 20 °C for 20 d was not significantly different because after 13 d, the mature fruit was over ripened thus lycopene levels had begun to decline. However, immature fruit stored at 20 °C was continually undergoing the ripening process, which was accompanied by gradual increases in lycopene levels of black tomatoes. These results agree with those of Choi et al. (2010), who showed that lycopene content decreased significantly in over-ripe tomatoes. The maximum amount of lycopene of black tomatoes was found in mature fruit stored at 12 °C for 13 d (192.6 mg kg^{-1}d.w.) (Table 4), and was half the amount that was found in typical red tomatoes stored at 12–14 °C for 15 d [85 mgkg^{-1}d.w.] (Gómez et al., 2009). Synthesis of carotenoids, particularly lycopene in the typical red tomato is also affected by cold stress, especially at chilling temperature (Gómez et al., 2009; Javanmardi and Kubota, 2006).

The β-carotene content of mature fruits at harvest was 12.8 mg kg^{-1} higher than that of immature fruit in black tomatoes; however, this difference was not statistically significant.
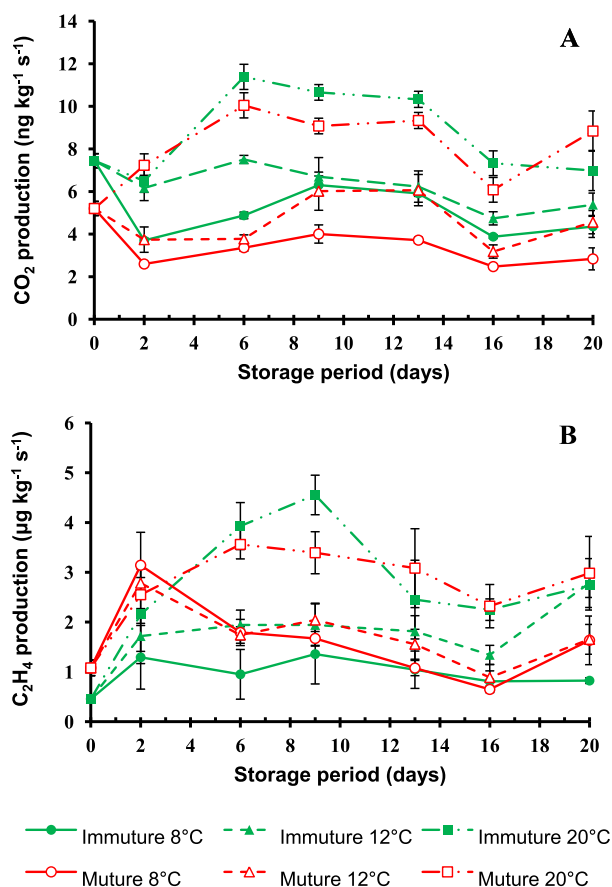
Figure 2 Changes in the respiration and ethylene production of black tomatoes during storage after harvest at immature (dark green color) and mature (conventional harvest stage of blackish red color) stages at different storage temperatures. -●; 8 °C, –▲; 12 °C, -··-■; 20 °C (immature) and -o; 8 °C, –△; 12 °C, -··-□; 20 °C (mature). Values represent the mean of 3 replicates, vertical bars represent standard error ($n = 3$). Data at day 0 represent the initial value recorded in black tomatoes before storage on the first day after harvest.

The β-carotene content in both stages with storage under all conditions increased and reached a peak at 6 d of storage before decreasing dramatically, reaching a nadir after 20 d of storage (Table 4). The extreme loss of β-carotene at 20 d of storage indicated an inappropriately long storage time. The maximum amount of β-carotene was found in both immature and mature black tomatoes stored at 12 °C for 6 d (183.4 and 188.7 mg kg^{-1}d.w., respectively) or one week before the peak of lycopene (Table 4). The β-carotene content of mature fruits remained high even after storage at 12 °C for 13 d, at which time the maximum content of lycopene was also observed. This means that 12 °C for 13 d is the optimum storage temperature and duration to obtain black tomatoes of high quality. However, the rate of β-carotene accumulation was the highest in the early stage of ripening (between mature green to yellow) and then decreased in orange and red ripe tomatoes, in contrast to lycopene accumulation, which continuously increased until the late stages of ripening (Riggi et al., 2008). In our experiment, the highest lycopene accumulation of black toma-

toes, which occurred 1 week after β-carotene accumulation, probably resulted from attenuated conversion of lycopene to β-carotene. Thus, β-carotene synthesis was partially blocked or reversed in low-temperature storage, leading to lycopene accumulation. The precipitous drop in β-carotene levels at 20 d post-storage occurred at all temperatures. β-carotene was possibly converted or isomerized into other derivative compounds including flavor and aroma constituents (Lewinsohn et al., 2005), or it was converted back into lycopene (Alba et al., 2000). In the mevalonate-independent pathway (Rohmer pathway) located in the plastids, plastid isoprenoids including plant growth hormones [gibberellins, abscisic acid, and plant pigments (carotenoids and chlorophyll)] are derived from isopentenyl pyrophosphate (IPP) (Lois et al., 2000). In addition, a previous report showed that the accumulation of lycopene in tomato fruits is favored at 20 °C, as the conversion of mevalonic acid into phytoene and lycopene was highest, whereas the conversion of lycopene to β-carotene was relatively slow. We found that β-carotene of black tomatoes was reversely convertible to lycopene, even at lower temperatures of 8 °C, 12 °C. Based on our findings, the dramatic loss of β-carotene followed by accumulation of lycopene clearly indicates that lycopene accumulation in black tomatoes is affected by storage temperature and maturation. Moreover, the loss of both lycopene and β-carotene after 20 d of storage suggests the conversion of this compound into other derivative compounds (Lewinsohn et al., 2005; Lois et al., 2000). Storage at 12 °C for 6–13 d is optimal for the production of β-carotene and lycopene in mature black tomatoes. In addition to the enhanced accumulation of lycopene with storage at 12 °C, heat treatment before storage also enhances lycopene content in tomatoes (Lurie and Klein, 1992). In contrast, the extreme loss of β-carotene and sustainable lycopene in mature black tomatoes resulted in little change in the lycopene/total carotenoid ratio, which was different from the case in typical red tomatoes. Typical red tomatoes showed a large change in lycopene and a minor change in carotenoid during maturation, resulting in a dramatic increase in the lycopene/total carotenoid ratio from the mature green stage to the red stage.

3.5. Chlorophyll

Table 5 shows the changes in the chlorophyll content of black tomatoes stored at different temperatures. In our experiment, the chlorophyll content of both immature and mature black tomatoes tended to decrease for 20 d under all storage conditions. This degradation was obvious at the higher temperature, 20 °C. Chlorophyll is obviously degraded enzymatically during tomato ripening, which is accompanied by a corresponding loss of the green color of the typical red tomato. In black tomatoes, the chlorophyll content of mature fruit stored at 12 °C for 20 d still remained as high as that of immature fruit stored at 20 °C and 12 °C, resulting in a slight greenish color skin color being retained in the mature fruit, even though the fruit appeared blackish red. However, the incomplete chlorophyll degradation in mature black tomatoes stored at 12 °C for 13 d helped to maintain the dark red skin color with high contents of both carotenoids and chlorophylls. At the end of storage at 8 °C in black tomatoes, immature fruits showed still higher chlorophyll content than mature fruits stored at all temperatures. Immature fruits stored at 8 °C failed to ripen

Table 3 Fruit skin color of immature (dark green color) and mature (conventional harvest stage of blackish red color) black tomatoes during storage at various temperatures for 20 d.

Maturity Stages	Storage Temp.	Storage period (days)						
		0	2	6	9	13	16	20
Hunter a* value								
Immature	8 °C		−10.1eC	−6.9dD	−3.7cE	−1.9bE	−0.7abD	0.5aD
	12 °C	−11.6B[z]	−8.8eC	−3.3dC	2.3bD	0.5cD	3.0bC	5.9aC
	20 °C		−9.1dC	−2.9cC	3.3bCD	3.7bC	4.7abC	6.8aC
Mature	8 °C		3.9cB	3.9cB	4.2cC	5.9bcB	6.8bB	9.2aB
	12 °C	6.7A	7.2cA	8.0cA	8.9bcB	10.5abA	10.5abA	12.5aA
	20 °C		7.2bA	9.3bA	12.3aA	12.4aA	12.0aA	13.5aA
Hunter b* value								
Immature	8 °C		20.9aA	18.2bB	15.4cBC	17.6bC	17.0bB	17.9bB
	12 °C	22.7A	19.5aBC	16.6bC	13.6dD	15.2cD	14.0dC	15.2cC
	20 °C		21.0aa	17.8bB	14.6cCD	14.2cD	13.9cC	14.7cC
Mature	8 °C		19.7abAB	19.4abA	18.7bA	19.1abAB	18.6bA	20.4aA
	12 °C	19.1B	17.2bcD	16.6cC	16.0cB	18.0bBC	17.0bcB	19.3aAB
	20 °C		18.2aCD	18.6aAB	18.9aA	19.7aA	19.8aA	19.5aA
Hunter a*/b* value								
Immature	8 °C		−0.48eC	−0.38dD	−0.24cC	−0.11bD	−0.04abD	0.03aD
	12 °C	−0.5B	−0.46eC	−0.20dC	0.20bB	0.03cC	0.21bC	0.38aC
	20 °C		−0.44dC	−0.16cC	0.24bB	0.26bB	0.33abBC	0.46aBC
Mature	8 °C		0.20cB	0.21cB	0.23cB	0.30bcB	0.36bB	0.46aB
	12 °C	0.4A	0.42dA	0.48cdA	0.55bcA	0.58bcA	0.61bA	0.66aA
	20 °C		0.40cA	0.50bA	0.65aA	0.63aA	0.60abA	0.68aA

[z] Means with the same uppercase letter in the column or same lowercase letter in the row were not significantly different at P < 0.05 with Duncan's multiple range test.

Table 4 Changes in lycopene and *β*-carotene content of immature (dark green color) and mature (conventional harvest stage of blackish red color) black tomatoes during storage at various temperatures for 20 d.

Maturity Stages	Storage Temp.	Storage period (days)				
		0	2	6	13	20
Lycopene (mgkg^{-1} dry weight)						
Immature	8 °C		85.3 ± 0.0aD	86.2 ± 0.0aC	89.9 ± 8.2aD	80.1 ± 14.1aC
	12 °C	50.5 ± 0.1B[z]	93.8 ± 0.0bC	100.8 ± 6.8abC	109.0 ± 15.7aD	90.5 ± 9.9bC
	20 °C		83.5 ± 3.5bD	97.9 ± 15.7bC	131.1 ± 14.2aC	125.4 ± 9.1aA
Mature	8 °C		105.6 ± 2.0 dB	131.7 ± 0.0bB	159.6 ± 16.9aB	118.1 ± 1.6cAB
	12 °C	105.5 ± 0.0A	129.2 ± 1.5cA	157.0 ± 4.1bA	192.6 ± 20.2aA	107.1 ± 9.0cB
	20 °C		106.9 ± 0.0 dB	138.5 ± 8.7bAB	161.3 ± 9.2aB	125.6 ± 3.4cA
β-carotene (mgkg^{-1}dry weight)						
Immature	8 °C		139.7 ± 4.1bAB	166.6 ± 6.1aA	108.8 ± 10.8cBC	31.8 ± 4.6dA
	12 °C	43.6 ± 5.7A	133.5 ± 5.9bBC	183.4 ± 6.6aA	120.9 ± 26.0bB	27.5 ± 2.9cAB
	20 °C		104.4 ± 26.6bD	170.0 ± 24.8aA	127.5 ± 38.5bB	26.1 ± 5.2cB
Mature	8 °C		118.8 ± 1.9bCD	167.1 ± 17.1aA	84.5 ± 4.2cCD	17.3 ± 2.9dC
	12 °C	56.4 ± 8.6A	157.7 ± 5.0cA	188.7 ± 8.3aA	164.3 ± 5.3bA	17.7 ± 0.5dC
	20 °C		132.7 ± 20.9bBC	161.3 ± 33.6aA	80.2 ± 0.3cD	15.0 ± 1.2dC
Lycopene/ Total carotenoids ratio						
Immature	8 °C		0.38	0.34	0.45	0.72
	12 °C	0.54	0.41	0.35	0.47	0.77
	20 °C		0.44	0.37	0.51	0.83
Mature	8 °C		0.47	0.44	0.65	0.87
	12 °C	0.65	0.45	0.45	0.54	0.86
	20 °C		0.45	0.46	0.67	0.89

[z] Means with the same uppercase letter in the column or same lowercase letter in the row were not significantly different at P < 0.05 with Duncan's multiple range test.

Table 5 Changes in total chlorophyll content (mg kg^{-1} fresh weight) of immature (dark green color) and mature (conventional harvest stage of blackish red color) black tomatoes during storage at various temperatures for 20 d.

Maturity Stages	Storage Temp.	Storage period (days)				
		0	2	6	13	20
Immature	8 °C		123.7 ± 3.5aB	101.0 ± 2.8cAB	113.6 ± 7.7bA	98.6 ± 0.2cA
	12 °C	203.5 ± 7.9A^z	125.8 ± 1.3aB	93.8 ± 5.5abAB	70.2 ± 46.9bB	80.4 ± 6.0abB
	20 °C		139.5 ± 5.4aA	94.6 ± 3.8bAB	91.1 ± 7.3bAB	68.9 ± 1.6cBC
Mature	8 °C		128.1 ± 1.6aAB	76.3 ± 30.7bB	84.1 ± 4.2bAB	76.0 ± 7.8bB
	12 °C	147.5 ± 10.1B	127.3 ± 8.0aAB	112.4 ± 32.7aA	118.8 ± 2.5aA	84.8 ± 19.9bAB
	20 °C		134.9 ± 8.4aAB	84.4 ± 11.3bB	68.5 ± 9.8bcB	52.3 ± 20.4cC

^{z} Means with the same uppercase letter in the column or same lowercase letter in the row were not significantly different at P < 0.05 with Duncan's multiple range test.

because of the failure of complete chlorophyll degradation. The loss of chlorophyll during storage occurred either through degradation during ripening or an increase in the conversion of mevalonate-derived IPP into more carotenoids, as both chlorophylls and carotenoids share the same precursor (Grierson, 1985; Lois et al., 2000). It is possible that low temperature affects the pathways that convert chlorophyll, such as the β-carotene synthesis pathway. Yahia et al. (2007) demonstrated that chlorophyll was degraded completely in tomatoes stored at 20 °C, whereas degradation was delayed in fruits stored at 4 °C.

4. Conclusion

In conclusion, the levels of carotenoids and chlorophyll, which are functionally important compounds and pigments in black tomatoes, are dependent on storage temperature. In black tomatoes, lycopene (a red pigment) accumulated rapidly in immature fruit stored at high temperature; this correlated with an increased Hunter index and was dependent on storage temperature and duration. Synthesis of β-carotene (a yellow pigment) was partially blocked or reversed upon storage at low temperature; this coincided with lycopene accumulation. Immature black tomato fruits failed to undergo further pigment changes during cold storage, especially at 8 °C, as the green chlorophyll pigment was not degraded. Mature black tomato fruits stored at 12 °C required 13 d of storage to obtain the optimal levels of lycopene, β-carotene and chlorophyll; these conditions also favored the best quality content and longest storability period. Thus, the levels of these pigment compounds could be suitable markers of black tomatoes quality and storability.

Acknowledgement

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