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Changes in carotenoid and chlorophyll content of black tomatoes (*Lycopersicon esculentum* 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.

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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 com-

pounds. Tomato ranked first as the major source of lycopene followed by β -carotene and vitamins C and E (Garcia-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 carotenogenesis 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 mg kg^{-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, Hampshire, UK). Fifty fruits per replicate were penetrated at a speed of $2 \text{ mm} \cdot \text{s}^{-1}$ 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 1L containers containing 3 fruits 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 ($10 \mu\text{L L}^{-1}$) 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.

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