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Investigation on the Cause(s) of Tomato Fruit Discoloration and Damage under Chilling Condition Using External Antioxidants and Hot Water Treatment

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Abstract: The color of tomato fruit turns to red from green during ripening. The accumulated lycopene content of red tomato fruits is reduced when the fruits stored in the refrigerator. Therefore, this study was conducted in order to investigate the reason why red color tomato turns to light red when the fruit is stored at chilling temperature. A tomato variety (cv Rotarno) grown under greenhouse condition and harvested at red stage was used to assess color and firmness. Before storage at these temperatures, red tomato fruits were infiltrated by different concentration of antioxidants (Vitamin C, L-galactonic acid- γ -lactone and Butylated hydroxyanisole (BHA) and also treated by hot water. The color and firmness of the fruits were measured by pigment analyzer and Zwick, respectively. Dessicator with vacuum pump was used for infiltration of antioxidants and water. Antioxidants were infiltrated through the scar of the fruits by applying 400 Mbar pressure for 15 sec. for each concentration. Red tomatoes were treated in hot water at 40, 45 and 50°C for 2, 5 and 10 min each. All antioxidant infiltrated and hot water treated fruits were stored at 4°C for three weeks. Statistically significant difference was not observed on color and firmness among different fruits treated by different concentration of antioxidants. Hot water treatment also showed no significant difference on color value between treated and non-treated fruits. However, hot water treated fruits scored significantly higher firmness than non-treated fruits. In general, the results showed that antioxidants as well as hot water treatment did not maintain accumulated lycopene content of the red tomato fruits which, stored at chilling temperature.

Key words: Tomato, lycopene, firmness, antioxidants, hot water treatment

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most important and extensively consumed vegetable crops worldwide, which is produced for its delicious fruit (Giovannucci, 1999; Sahlin *et al.*, 2004). As tomato is a tropical crop by its origin, the fully matured but green fruit is stored at a relatively warmer temperature to avoid injury from chilling (Guilleu *et al.*, 2006). Consumers prefer tomato with green or light pink color extending on the cultivars, but the fast acceleration change related to ripening can be a limitation to marketing (Guilleu *et al.*, 2006). Tomato contains metabolites like carotenoids (especially lycopene), a compound known with its antioxidant property in preventing some kinds of diseases such as cancer and coronary heart disease. Moreover, it is also useful for its β -carotene and vitamin C contents (Abushita *et al.*, 2000; Toor and Savage, 2006). Antioxidants are known for their effect of inhibiting or delaying the oxidation of vital molecules like lipids

through inhibiting the oxidative chain reaction before initiated (Javanmardi and Kubota, 2006). It has been reported that the antioxidant content of tomato is a function of genetic and environmental factors as well as the ripening stage (Javanmardi and Kubota, 2006). Antioxidant compounds are vital in determining tomato fruit nutritional quality (Frusciant *et al.*, 2007).

The color of tomato turns to red from green during ripening. This red color of the fruit is provided by the carotenoid pigment lycopene that gives appearance to the fruit and one of the most important attribute of the tomato fruit. It is influenced by the genetic make-up of the plant and its growing environment (Kabelka *et al.*, 2004). In red tomato fruits, the carotenoids often happen in the form of lycopene (Kabelka *et al.*, 2004). As the color of the tomatoes is governed by genetic factor, tomatoes with gene encoding for high pigment have increased total carotenoid levels and appear deeper red in color due to an increase in chromoplasts (Kabelka *et al.*, 2004). Like the genetic factor, the color development is also influenced

by environmental factors like temperature. While the development of red color is enhanced by high temperature, chilling inhibits the development of the color (Verlinden *et al.*, 2004).

Lycopene is a biologically active carotenoid compound found in many fruit and vegetables responsible for the characteristic red color. Tomato fruits are major producers of lycopene. Lycopene has so far been reported to have association to several health benefits (Agarwal *et al.*, 2001) because of a highly conjugated structure, contributing to its ability to quench singlet oxygen (reduce oxidative stress problem) more efficiently than any other carotenoids commonly found in the diet (Hadley *et al.*, 2003). The ripening of tomato fruit is highly organized process during which color and other quality characters change in a very coordinated manner. One of the most obvious uniqueness of ripening of tomato is striking increase the carotenoid content of the fruit. The formation of red color is because of the huge accumulation of lycopene within the plastids and loss of chlorophyll (Bramley, 1997). Lycopene content is liable to loss up on processing and storage temperature; under different food processing conditions, lycopene undergoes degradation by isomerization and oxygenation, which impairs its bioactivity and reduce functionality for health benefits (Xianquan *et al.*, 2005). Tomato stored at high temperature develops a bright red color than tomatoes put in refrigerators, it is due to the autoxidation of lycopene under chilling temperature will lead to fragmentation of molecules which causes noticeable and irreversible color loss (Xianquan *et al.*, 2005; Toor and Savage, 2006). Firmness is one of the parameter usually used to assess fruit quality. It has a direct association to fruit development, ripening and shelf life (De Ketelaere *et al.*, 2004) and therefore, the magnitude of fruit firmness is used as an indication of fruit quality (Batu, 2004). Firmness is probably the final and most reliable parameter depending on which consumers make decision to buy a given tomato fruits because consumers believe the higher the firmness the better eating quality (Batu, 2004). Fruit firmness is inversely proportional to ripening in tomato as tomato pericarp discs decrease in firmness during color change in relation to that seen in intact fruit. It is also reported that the firmness of tomato pericarp discs has strong association with skin and flesh color (Campbell *et al.*, 1990). Loss of firmness during ripening, in storage, while marketing and distribution of tomato fruits can be a major problem because it may make the fruits liable to post harvest damage (Batu, 2004).

Temperature is a key factor determining the quality of tomatoes during postharvest handling and management. (Verlinden *et al.*, 2004; Yang *et al.*, 2009). Development of

red colour and softening of tomato fruit deepens on temperature. The optimal temperature for red colour development on tomato fruit is reported to be ranging between 16-26°C (Mostofi and Toivonen, 2006). Tomato fruits are tropical by their origin and hence are susceptible to chilling injury when stored temperature below 12.5°C (Lurie *et al.*, 1996; Yang *et al.*, 2009). However, according to the study of Whitaker (1991) partial ripening of tomatoes showed a decreased sensitivity to chilling temperature.

Typical symptoms of chilling injury in tomato fruits include failure to develop red colour, skin lesions, quick softening relative to that of non-chilled fruit, ion leakage and susceptibility to decay (McDonald *et al.*, 1999; Yang *et al.*, 2009). Antioxidant activity of tomato fruits is higher at low temperature than high temperature. This depends on several factors including genetics and environmental conditions (Javanmardi and Kubota, 2006). Increasing antioxidant activity for the period of low temperature storage is related to ripening process and metabolism of phenolic compounds. Moreover, low temperature induces free radicals in product, this leads to increase phenolic metabolism and antioxidant activity at storage (Javanmardi and Kubota, 2006). The antioxidant activity of phenolic compounds are attributed to their redox properties, which helps in adsorbing and neutralizing free radicals, quenching singlet oxygen and triplet oxygen or decompose peroxide. Tomato fruit firmness continuously decreased during storage and post ripening was seen to have good association with increased temperature. Stored at the chilling temperature firmness was seen to be maintained because of the fact that softening was inhibited during post storage ripening of tomato stored under chilling temperature. Tomato stored at 12°C had acceptable firmness for commercial purposes after post storage ripening (Artes and Escriche, 1994). Even though, storage at low temperature is a common practice to slowdown softening of the tomato fruit, the reverse can happen at low temperature as a result of chilling injury (Lana *et al.*, 2005).

Hot water treatment before the fruits stored at chilling temperature is important to reduce chilling injury (McDonald *et al.*, 1999). Hot water treatment was better than air treatment in reducing chilling injury (Lurie *et al.*, 1997). According to Yang *et al.* (2009), heat treatments that increase chilling tolerance are thought to work through the induced synthesis and accumulation of specific Heat Shocked Proteins (HSPs). Yang *et al.* (2009) reported that these proteins confer thermo-tolerance on the tissue in which they are formed and hence subsequent exposure to chilling temperature does not cause damage. The study of Soto-Zamora *et al.* (2005) indicated that

green-mature cherry tomato fruits exposed to hot air at 34°C for 24 h prior to storage at 10°C for up to 30 days showed the least losses in antioxidant content and fruit colour developed adequately.

According to previous studies, when red tomato fruit is stored in the refrigerator the red colour turns to light red. This might be because of reduction of lycopene. This is believed to be because of the fact that the free radical production becomes high and hence maximum antioxidant activity at chilling condition. Lycopene is known for its antioxidant activity. And in order to neutralize the free radicals induced due to chilling temperature some amount of lycopene will be utilized for alleviating oxidative problem instead of making color. Therefore, the objectives of this experiment were to investigate the reason why red tomato fruits turn to light red when they are stored at chilling temperature and to see the effect of antioxidants (Vitamin C, L-galactonic acid- γ -lactone and Butylated hydroxyanisole) and hot water treatment on color and firmness of tomato fruit under chilling temperature.

MATERIALS AND METHODS

Experimental materials: Freshly harvested, non-damaged, red stage and round shaped truss tomato fruits with 60-116 g weight were used as a test material. According to the USDA standard tomato colour classification chart (USDA, 1991), "red" refers to more than 90% of the surface in aggregate shows red color which contains 3,165 mcg of lycopene in one fresh tomato. Roterno cultivar was used and this experimental cultivar grew in the Southeastern part of The Netherlands in greenhouse Rijkzwaan by using artificial light. Fifteen tomato fruits were used per treatment and each tomato was given a unique number to avoid mix-up in data recording and during repeated quality parameters measurement at different storage time.

Experimental setup and treatments: The experiments were carried out in the laboratory of Horticultural Production Chain group at Wageningen University from March 2011 to April 2011. Two separate experiments were set. Experiment one contained three antioxidants, water (0.5 mL) and non-treated (control) as a treatment. The antioxidants are vitamin C, L-galactonic acid- γ -lactone and Butylated hydroxyanisole each anti-oxidant having three different levels of concentration (60 mg/0.5 mL, 30 mg/0.5 mL and 15 mg/0.5 mL of water for Vitamin C), 0.895 mg/0.5 mL, 0.099 mg/0.5 mL and 0.0009 mg/0.5 mL of water for L-galactonic acid- γ -lactone) and (0.05 mg/0.5 mL, 0.025 mg/0.5 mL and 0.0125 mg/0.5 mL of

water for BHA). The second experiment used hot water as a treatment differing treating temperature and duration. Three temperatures (40, 45 and 50°C) were assigned as a level each having three level of duration of treating fruits (2, 5 and 10 min) and a control (non-treated). All antioxidants and water were infiltrated through the scar of fruit by using pipette inside the vacuum desiccator. Then after, the vacuum desiccator was connected via a valet to vacuum pump and 400 Mbar pressure for 15 sec to suck the air from the fruit and slowly release the vacuum, allowing chemicals and water to enter the fruit. After infiltration by antioxidants and water and also hot water treatment, the fruits were stored at 4°C for three weeks. During the storage time, color and firmness were measured at four days interval in order to see variations among the treatments in chilling conditions.

Parameters taken

Color: The color of the fruit was measured without destructing using CP pigment analyzer PA 1101. The method of Rutkowski *et al.* (2008) was employed after modification to collect a spectral from pigment analyzer in order to calculate normalized anthocyanin index [NAI $(I_{780nm} - I_{550nm}) / (I_{780nm} + I_{550nm})$] to measure redness. This indicated the color loss variation in tomato fruits stored at chilling temperature. In order to measure spectral value (in nanometer) from stored fruits three circular spots were made on the surface of fruits making the first on the blossom end where fruit number was written. The remaining two spots were assigned as spot 1 and spot 2 from which color was measured and averaged.

Firmness: Firmness was also measured without destructing the fruits using Zwick Universal Type Machine (Lana *et al.*, 2007). In this method, the tomato fruit was placed in a plastic ring with its stalk positioned perpendicular to the center of gravity. The firmness was determined by exerting maximum force to compress the tomato fruit down to 2 mm at 40 mm/min speed from lowering the probe until it touches the tomato skin. One spot was used from a fruit to measure the firmness repeatedly from each treated and non treated fruit until the end of experiment.

Data analysis: Analysis of variance for the repetitive measurement was used to determine variation among the treatments for the variables recorded. The data obtained from this experiment was analyzed using GenStat 13th Edition (VSN International, 2010). The results from the analyzed data were used to observe variations existing among different treatments.

RESULTS

Effect of antioxidants and water infiltration on color and firmness of red tomato fruit stored at 4°C: The red tomato fruits infiltrated by different types and concentration of antioxidants, water and control (non-infiltrated) fruits indicated that similar trend of declining in color values at 4°C storage (Fig. 1a-c). The fruits which were infiltrated by highest concentration of vitamin C (60 mg/0.5 mL) significantly ($p < 0.05$) decreased in NAI (Normalized Anthocyanin Index) values from beginning of experiment throughout storage time than the fruits infiltrated by the lowest concentration of vitamin C (30 and 15 mg/0.5 mL) (Fig. 1a). Moreover, the fruits infiltrated by different concentrations of L-galactonic acid- γ -lactone and BHA did not show significant difference among the treatments unlike vitamin C infiltrated fruits (Fig. 1b-c). On the other hand, control fruits scored highest values of NAI than all antioxidants and water infiltrated fruits (Fig. 1a-c).

The fruits infiltrated by different concentration of antioxidants, water and control fruits stored at 4°C were

showed reduction in firmness during storage (Fig. 2a-c). Fruits infiltrated by highest concentration of vitamin C (60 mg/0.5 mL) showed a pronounced decline of initial firmness than lowest concentration of vitamin C (30 mg/0.5 mL and 15 mg/0.5 mL), water infiltrated fruits and control fruits (Fig. 2d). Different concentration of L-galactonic acid- γ -lactone and BHA infiltrated fruits did not show observable differences in loss of firmness unlike vitamin C (Fig. 2a-c). Even though, there was a small variation in firmness among treatments, they were not significantly differing to each other.

Effect of hot water treatment on color and firmness of red tomato fruit stored at 4°C: Reduction of accumulated lycopene content in hot water treated (40, 45 and 50°C for 2, 5 and 10 min each) and control (non-treated) red tomato fruits held at 4°C were observed (Fig. 3a-c). However, the result did not show significant difference in reduction of NAI (color values) among the treatments (Fig. 3a-c).

Hot water treated and control red tomato fruits stored at 4°C were also exhibited a reduction in firmness

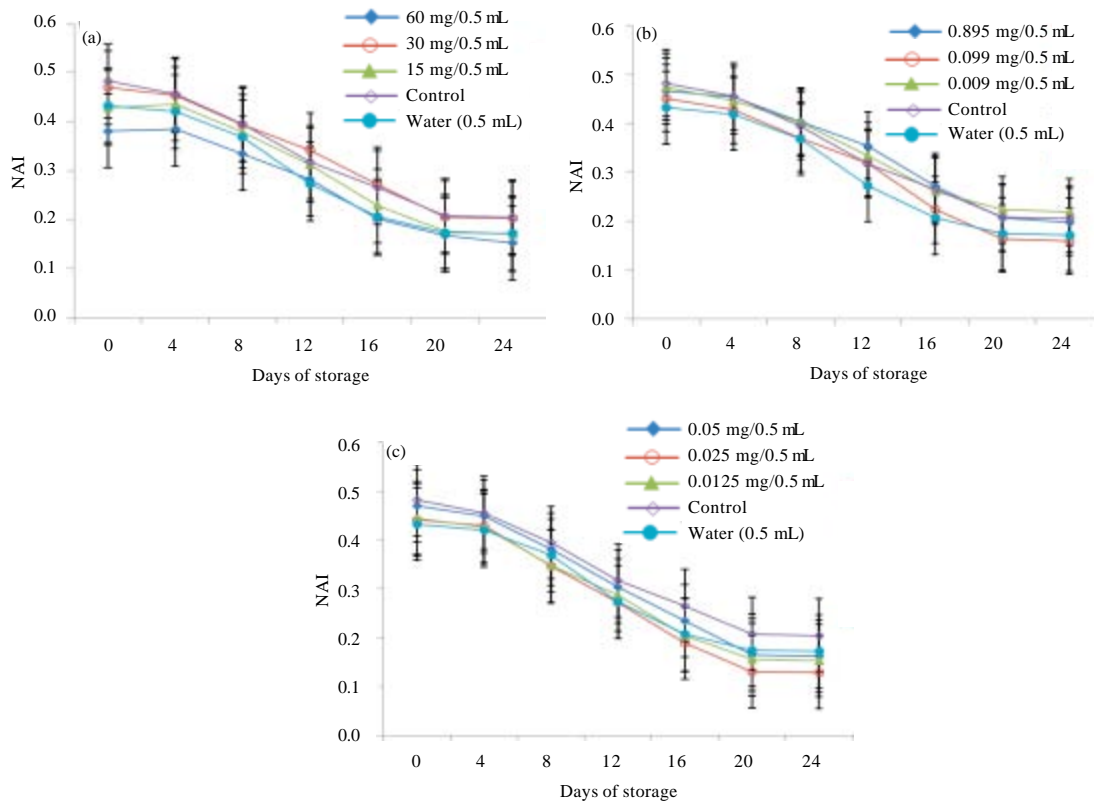


Fig. 1 (a-c): Color of red stage tomato fruits during storage at 4°C, infiltrated with (a) Vitamin C, (b) L-galactonic acid- γ -lactone and (c) Butylated hydroxyanisole and water, NAI: Normalized anthocyanin index, Data are Means \pm SE

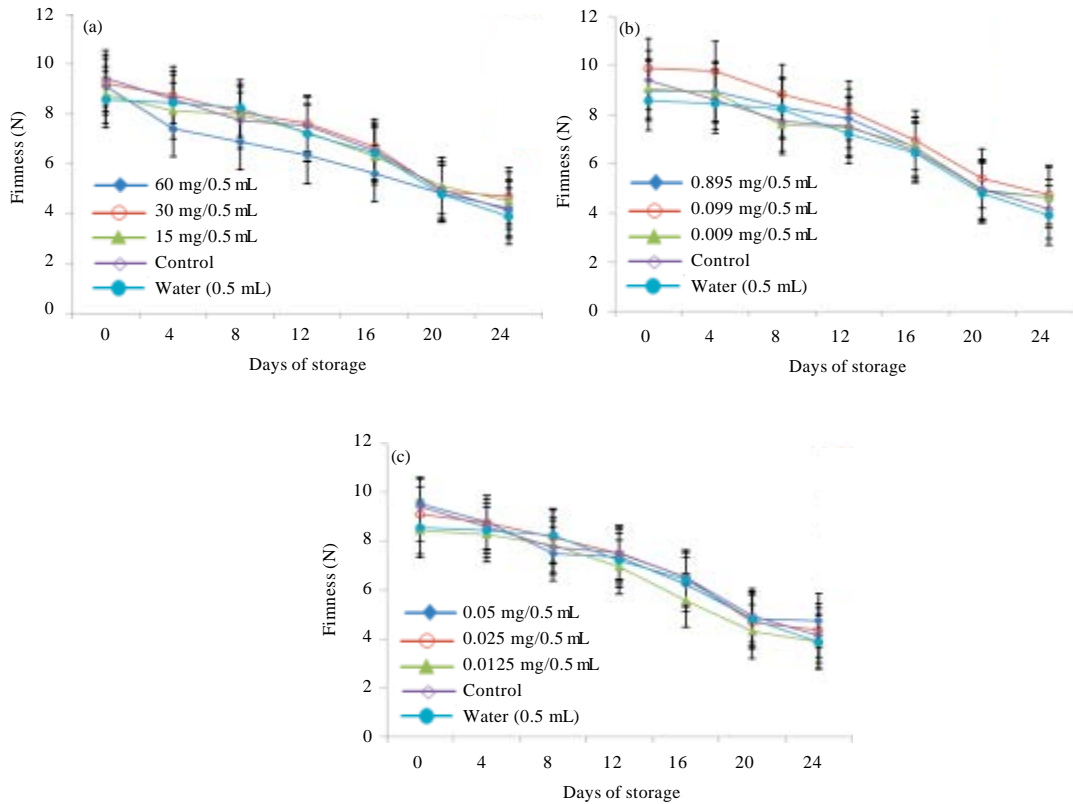


Fig. 2(a-c): Firmness of red stage tomato fruits during storage at 4°C, infiltrated with (a) Vitamin C, (b) L-galactonic acid-γ-lactone and (c) Butylated hydroxyanisole and water, Data are Means±SE

(Fig. 4a-c). Hot water treated fruits (40, 45 and 50°C for 2, 5 and 10 min each) did not show significant differences on firmness among themselves (Fig. 4a-c). However, differences were observed between hot water treated and control fruits after a certain days of storage. Control fruits were significantly ($p < 0.05$) softer than hot water treated fruits after day 12, especially 40 and 45°C for 2, 5 and 10 min each treated fruits (Fig. 4a and 4c). The red tomato fruits did not show observable variation in firmness due to longer time treatment at all temperatures. However, the fruits treated by high temperature (50°C) scored lower firmness at the end of storage time than low temperature treated fruits (Fig. 4c).

DISCUSSION

Lycopene content accumulated in red tomatoes was declined when fruits were stored at chilling temperature (this case 4°C). There is a report mentioning that low temperature reduces the accumulated lycopene content of the tomato fruits (Soto-Zamora *et al.*, 2005; Xianquan *et al.*, 2005; Toor and Savage, 2006).

Javanmardi and Kubota (2006) reported that low temperature induces free radicals in product leading to increase in phenolic metabolism and antioxidant activity at storage. As lycopene is antioxidant by itself and in order to neutralize the free radicals in response to chilling injury, the lycopene content decreases because some amount will be utilized for alleviating oxidative problem instead of making colour. According to the study of (Javanmardi and Kubota, 2006), tomato fruit stored at 5°C inhibited lycopene enrichment and increased antioxidant activity.

All antioxidants infiltrated fruits were observed in decreasing color value of the red tomato fruits during storage. This is probably due to the fact that infiltrated antioxidants were not effectively distributed throughout the fruits to maintain lycopene content of red tomato fruits stored at low temperature. Besides, the losses of accumulated lycopene in red tomato fruits might not be due to the chilling injury problem. This is in agreement with the result of Lurie *et al.* (1996). They reported that, when chilled fruits were transferred to 20°C, the mRNA level involved in lycopene (pTOM5) and ethylene

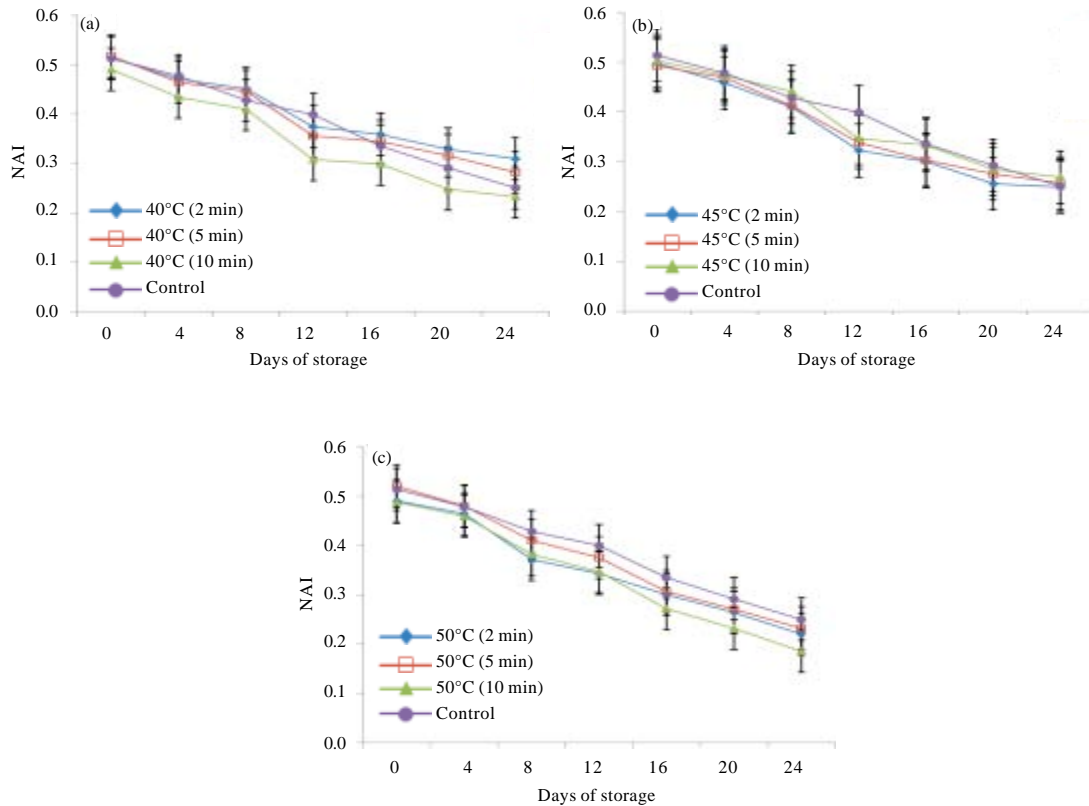


Fig. 3(a-c): Color value of the red tomato fruits treated with hot water by different temperature scales (a) 40, (b) 45 and (c) 50°C and stored at 4°C for different days, NAI (Normalized Anthocyanin Index), Data are Means±SE

(pTOM13) synthesis pathway increased in tomato fruits. Moreover, the lycopene content (red color development) of the fruits also increased. So, the reduction of accumulated lycopene might be related to suppression of carotenoid biosynthesis pathway due to low temperature, as lycopene is one component of carotenoid biosynthesis pathway. According to the study of Javanmardi and Kubota (2006) the temperature has a tremendous effect on lycopene development. It has been also reported by the same authors that the formation of lycopene depends on the temperature range and seems to occur between 12 and 32°C. Therefore, the reduction of accumulated lycopene content during storage of tomato fruits at chilling temperature (in this case 4°C) might not be due to elimination of carotenoid and ethylene biosynthetic pathway by chilling injury, but might be because of the reason that the pathway facilitator mRNA is suppressed due to chilling temperature. Those messengers are known to play a significant role to activate pathways that are involved in color development and ethylene production.

The softening rate of fruits stored at chilling temperature especially (2-8°C) was lower than the fruits stored at non-chilled temperature (Lana *et al.*, 2005). Tomato is a chill-sensitive fruit in which chilling response may be classified according to the extent of damage (Hobson, 1987). Thus, reduction in the firmness of tomato fruits stored at low temperature can happen because of chilling injury.

A study by McDonald *et al.* (1999) and Soto-Zamora *et al.* (2005) revealed that hot water treatment increased respiration and ethylene production. In addition, red color development is enhanced by hot water treatment during ripening of green mature tomatoes and the process is inhibited by chilling. According to the report by Yang *et al.* (2009) hot water treated mature green tomatoes had higher color values than non-treated fruits, which indicates that exposure of green tomato to higher temperature induces the reddening of the fruits.

In our study, red tomato fruits treated by 40, 45 and 50°C for 2, 5, 10 min each could not maintain accumulated

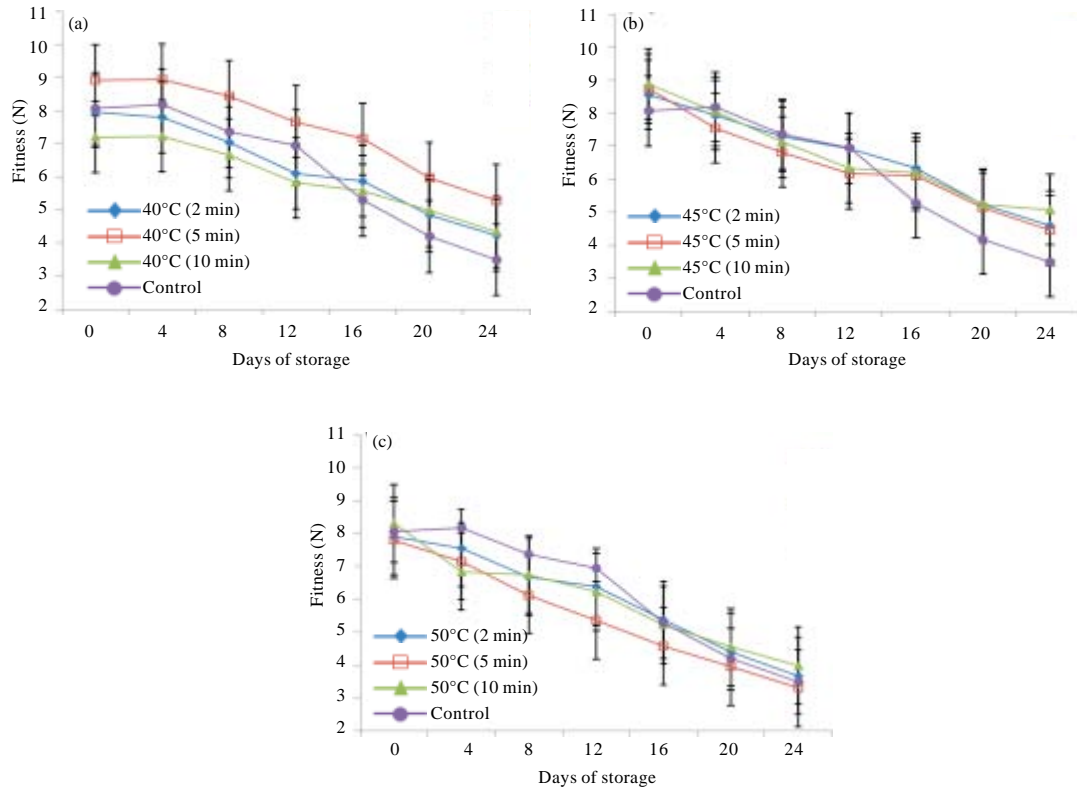


Fig. 4(a-c): Firmness of the red tomato fruits treated with hot water by different temperature scales (a) 40, (b) 45 and (c) 50°C and stored at 4°C for different days, Data are Means±SE

lycopene content during storage at 4°C. All hot water treated fruits in different scale of temperature and time decreased the already accumulated lycopene content during storage. According to above studies, this might be due to the fact that hot water treatment has more effect in green mature tomatoes than red tomatoes for degradation of chlorophyll and synthesis of lycopene when the fruits stored at chilling temperature because these processes would otherwise be inhibited by chilling. Besides, Tuan *et al.* (2004) reported that tomato fruits had an orange color instead of red as a result of high heat treatment stimulating the change of lycopene to beta-carotene. Hence, hot water treatment for fruit that already reached to climacteric picks (red tomato fruit) does not give satisfactory increment in synthesis of lycopene and maintaining of accumulated lycopene when hot water treated red tomato fruits stored at chilling temperature.

In this study, the firmness of control fruits decreased after day 12 than hot water treated fruits. This finding is similar to that of Lurie and Klein (1992) which showed that the firmness of heated tomatoes was not significantly less or was even greater than that of the non-treated ones

right after heat treatment. The difference in firmness between treated and non-treated fruits might be due to the protective treatments such as hot water treatment and heat shocks that were applied before chilling are thought to make tissue more resistant to the physiological damage that occurs during chilling. Moreover, the fruits treated by applying higher temperature (50°C) scored lower firmness than the fruits treated by lower temperature (40 and 45°C) at the end of experiment. This might be because of the ripening stage of the fruits used in this experiment, which were red ripe tomatoes. Normally, red tomatoes are less resistant to heat than green matured tomatoes. Therefore, hot water treatment of red tomatoes by applying higher temperature causes more softening of the fruits than the red fruits treated by hot water applying low temperature.

In general, this experiment was conducted with the assumption that lycopene would be destroyed as a result of chilling injury i.e., lycopene would be involved to scavenge free radicals as a result of chilling injury and its content is reduced. However, the result showed that the addition of different concentration of external antioxidants to help the scavenging process and hot water treatment

for synthesis and accumulation of heat-shocked proteins to increase chilling tolerance did not avoid the reduction of the lycopene content. Under chilling condition, the lycopene content in red tomato fruits is reduced as a result of suppression of the pathways of lycopene synthesis seems more plausible. Therefore, further investigation should be done to know which gene (s) is (are) suppressed (down regulated) in the lycopene synthesis pathway under chilling temperatures.

CONCLUSION

The study confirmed that infiltration of the red tomato fruits by antioxidants was not effective to maintain the accumulated lycopene content when the fruits were stored at chilling temperature. This study also showed that hot water treatment of red tomatoes in different scale of temperature and time before the fruits stored at chilling temperature did not help to maintain already accumulated lycopene content in the fruit. However, it helps to maintain the firmness of the fruits.

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