

*Full Length Research Paper*

# Effect of hot water treatment on reduction of chilling injury and keeping quality in tomato (*Solanum lycopersicum* L.) fruits

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Chilling injury is a physiological disorder caused by the exposure of fruits and vegetables to low temperature above the freezing point. Chilling can delay fruit ripening in tomato fruits. The objective of this study was to determine the effect of hot water treatment on reduction of chilling injury and keeping quality of tomato fruits. The experiment was done in post-harvest physiology laboratory of Jimma University using Complete Randomised Design (CRD) arrangement of treatments replicated three times. The experiment had three treatments: green mature tomato treated in water at 40°C and 50°C both for 20 min and control (non-treated) fruits. Results have indicated that 40°C treatment for 20 min resulted in reduced weight loss and chilling injury index but increased fruit firmness during storage. Moreover, shelf life was better than control by three and half days when fruits were treated by hot water at 40°C for 20 min. With regard to chemical quality attributes, 50°C treatment for 20 min was better for higher lycopene content compared to other treatments. Significant differences were not detected among the treatments for total soluble solids, pH and  $\beta$ -carotene. Hence, hot water treatment before storage can alleviate chilling injury and improving some quality characteristics of tomato fruits.

**Key words:** Chilling injury, chlorophyll, hot water treatment, lycopene, quality, tomato.

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important agricultural commodity worldwide. Tomatoes and tomato-based products are considered as healthy foods for several reasons. They are low in fat and calories, cholesterol-free, and good source of fiber. In addition, tomatoes are rich in vitamin A and C,  $\beta$ -carotene, lycopene and other antioxidants (Yahia et al., 2007). However, they are chilling sensitive at temperatures

below 10°C if kept for longer than two weeks or at 5°C for longer than six to eight days (Suslow and Cantwell, 2002 cited in El Assi, 2004).

Ethylene, in association with other hormones, plays a key role in the ripening process of climacteric fruits like tomato (Chaves and Mello-Farias, 2006). The ripening process involves decreasing firmness as a result of structural changes in the principal cell wall components

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that is, cellulose, hemicellulose and pectin. It also involves the accumulation of sugars such as glucose, fructose and organic acids in vacuoles and the production of complex volatile compounds that are responsible for the aroma and flavor of the fruit.

Chilling injury is a physiological disorder caused by the exposure of fruits and vegetables to low temperature above the freezing point (Soto-Zamora et al., 2005). Consequences of chilling injury are failure to ripen and develop full color and flavor, irregular color development, premature softening, surface pitting, browning of the seeds and increasing decay (Verlinden et al., 2004). Moreover, according to Lelièvre et al. (1997), chilling injury interferes with gene expression for ethylene biosynthesis depending on species, cultivar, developmental stage as well as duration of the chilling treatment. In addition, cell ultrastructure is altered following prolonged chilling (Kratsch and Wise, 2000). These symptoms appear when the fruits are transferred to non-chilling temperature (above 10 to 13°C) (Wang, 1994). Another study reported that tomato showed less sensitivity to chilling injury as ripening proceeds and ethylene production increases (Ben-Amor et al., 1999).

The extent of chilling injury is related to the duration of exposure to a particular temperature, post-chilling conditions, pre-temperature treatment, harvesting stage, degree of ripeness of fruits and cultivar (Saltveit, 2005). Changes in surface colour of mature green fruits are not correlated with the sensitivity of cultivars to chilling. However, within the standard and cherry (very small size) types, chilling-tolerant fruits change surface colour when subjected to chilling unlike their chilling-sensitive counterparts when picked early in the season. This shows that early harvested standard and cherry types are less sensitive to chilling effect when harvested early (Dodds et al., 1991).

Hot water treatment is considered to be better than air treatment in reducing chilling injury (Lurie et al., 1997). According to Zhang et al. (2005), heat treatments that increase chilling tolerance are thought to be related with induced synthesis and accumulation of specific heat shock proteins (HSPs). Krishnan et al. (1989) reported that these proteins can cause 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 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 loss in antioxidant content and fruit colour developed adequately.

In addition, studies have shown that subjecting tomato fruits to heat-shock treatments prior to chilling reduces the incidence and severity of chilling injury, reduces skin damage during storage, extends shelf life and inhibits ripening processes (Lu et al., 2010). Tomato fruits that are heat-treated with warm air at 38°C for 2 to 3 days can be kept for one month at 2°C without being affected by

chilling injury (Sabehat et al., 1996). However, some tomato quality attributes can be negatively affected by hot water treatment. For instance, pre-heating (at 38°C or higher) inhibits lycopene synthesis in tomatoes (Whitaker, 1994). Lycopene content was more than eight-fold higher in pericarp from non-preheated tomatoes compared with heat-treated tomatoes (Whitaker, 1994). This was attributed to the inhibition of transcription of mRNA for lycopene synthase, a key enzyme in the pathway (Sabehat et al., 1996) but the tomato recovers after heat removal (Lurie et al., 1996). Heat-treated tomatoes showed a wide variation in ripening stage ranging from breaker to orange whereas non pre-heated tomatoes were uniformly red ripe (Whitaker, 1994).

The previous works mentioned above in the field revealed the effect of higher temperature treatment on the quality where the treatments were limited to about 40°C with treatment durations from several hours to a few days. However, from practical point of view, treating tomato fruits for several hours or a few days would demand more resource if it is considered in large scale. Therefore, there was a gap in information about treatment of fruits with relatively higher temperature than previously proposed (for example about 50°C) and shorten the time of treatment. In addition, previous studies about the effect of the temperature treatment on chilling injury lack information on how the quality attributes of fruits were affected. Therefore, the objective of this study was to determine the effect of hot water treatment on chilling injury and quality attributes of tomato fruits when they are stored for longer period of time after treatment.

## MATERIALS AND METHODS

### Study area

The study was conducted in post-harvest physiology laboratory of Jimma University College of Agriculture and Veterinary Medicine.

### Experimental material and treatments

Tomato fruits of local cultivar 'Cochoro' grown in greenhouse at Jimma University College of Agriculture and Veterinary Medicine were harvested at the mature green stage. Fruits were dipped in water at 40 and 50°C for 20 min, excessive water was drained off and fruits were dried in cold air. Both hot water treated fruits and non-treated (control) fruits were then packed in polyethylene film bags and stored at 4°C for 16 days and then were transferred to 20°C and stay there for 16 days to allow ripening. Samples were removed every 4 days during the ripening period for analysis. Each treatment had 20 fruits and was replicated three times.

### Data collection

#### *Chlorophyll, carotenoids and lycopene content*

The content of chlorophyll, lycopene and carotenoids pigments were extracted by mixing 1 g of extracted tomato juice with 15 ml

acetone and hexane mix solution (4:6 ratio) at once, then the absorbance of the supernatant containing those pigments were measured at 663, 645, 505 and 453 nm using spectrophotometer at the same time. From these values the content of chlorophyll, lycopene and  $\beta$ -carotene in tissues were estimated by the following Equations 1, 2 and 3:

$$\text{Chlorophyll(mg/100 ml)} = 0.999A_{663} - 0.0989A_{645} \quad (1)$$

$$\text{Lycopene(mg/100 ml)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (2)$$

$$\beta\text{-carotene(mg/100 ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (3)$$

Where,  $A_{663}$ ,  $A_{645}$ ,  $A_{505}$  and  $A_{453}$  are the absorbance at 663, 645, 505 and 453 nm, respectively.

### Color of the fruits

During storage, tomato fruits were taken at specified time intervals for color measurements ( $L$ ,  $a$  and  $b$  values), which were measured with colorimeter (model: ACCUprobe, HH06, USA). It was calibrated using white reference plate ( $a = -409$ ,  $b = 867$ ,  $L = 8269$ ). Tomato fruits were scanned for color at two different locations to determine the average  $L$ ,  $a$  and  $b$  values during colorimetric measurements. Then color index, chroma and hue angle were calculated from  $L$ ,  $a$  and  $b$  values scale during storage by using the following Equation 4, 5 and 6:

$$CI = \frac{21.6a - 7.5b}{L_a} \times 100 \quad (4)$$

Where CI= color index

$$\text{Chroma} = (a^2 - b^2)^2 \quad (5)$$

$$\text{Hue angle} = \tan^{-1}(b/a)^2 \quad (6)$$

### Firmness

The firmness of the fruits was measured by using texture analyzer (model: TA.XT. plus). The firmness was determined by the maximum force exerted to compress the tomato fruit down to 5 mm at 10 mm/s speed from lowering the probe until it touched the tomato skin.

### Weight loss

Weight loss during post-harvest storage was determined by subtracting sample weights from their previous recorded weights and presented as percentage of weight loss compared to initial weight using the following Equation 7.

$$\text{Weight loss(percent)} = \frac{\text{Initial weight of tomato (g)} - \text{weight after interval (g)}}{\text{Initial weight of tomato (g)}} \times 100 \quad (7)$$

### Chilling injury

Chilling injury index (CII) was visually assessed by the scale of skin lesion that was estimated as percentage of affected surface area where 0 = no injury (no signs), 1 = slight (<20% of surface area), 2 = moderate (20-50% of surface area), and 3 = severe (>50% of

surface area). CII was calculated using Equation 8.

$$CII = \frac{(\sum(\text{scale} \times N))}{\text{total fruit number}} \quad (8)$$

Where N is the number of fruits on the corresponding scale.

### Shelf life

Shelf life of the fruits was recorded in days at 30% spoilage level on percent basis.

### Total soluble solids (TSS)

Total soluble solid was measured from the already extracted tomato juice using hand refractometer (model: 45-02).

### Titrateable acidity (TA)

Tomato juice was extracted from the sample with a juice extractor and clear juice was used for the analysis of TA by the methods described by Maul et al. (2000). Finally, the percentage acidity was determined by using the following Equation 9:

$$\text{Percentage of acid} = \frac{\text{Titer} \times 0.0064 (\text{citric acid factor})}{1 \text{ ml juice}} \times 100 \quad (9)$$

### pH

The pH value of tomato juice was measured by pH meter. To determine the pH value of tomato juice, the probe and meter was calibrated following the manufacturer's instruction. The pH measurement of each sample was read from the probe according to the manufacturer's specifications.

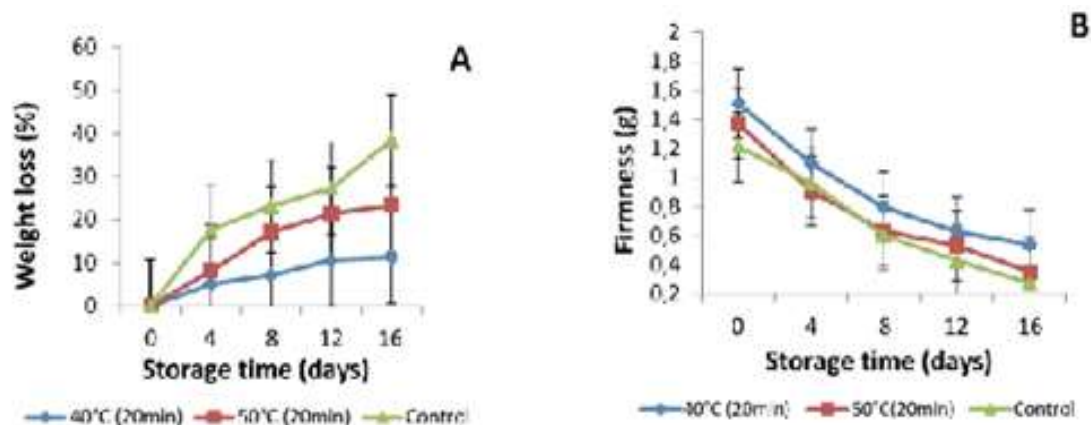
### Data analysis

All data were analyzed using GenStat statistical package 14<sup>th</sup> Edition (VSN International, 2012). Analysis of variance (ANOVA) was used to determine variations among the treatment effects for the variables recorded.

## RESULTS AND DISCUSSION

### Effect of hot water treatment weight loss and firmness of the fruit

There was a significant ( $p < 0.05$ ) difference in weight loss between control and hot water treated (40 and 50°C for 20 min) fruits. Weight loss of fruits in the treatments gradually increased from 0 to 11.2%, 23.3 and 38.4% in 40, 50°C and control treated fruits, respectively during the 16 days storage time (Figure 1A). 40°C treatment for 20 min generated the least weight loss compared to control (12.1% higher) and 50°C treatment for 20 min (27.2% higher). According to Lurie et al. (1997) the outer pericarp tissue of hot water treated fruits tomatoes had higher phospholipids, lower sterol contents, less saturated fatty



**Figure 1.** Effect of hot water treatments on weight loss (A) and firmness (B) of the fruit during storage at 20°C. Data are means  $\pm$  SE. Control (—▲—), 40°C (20min) (—◆—) and 50°C (20min) (—■—).

acid than unheated fruits, and this might be to the reason for the treated fruit to have less weight loss compared to control. The basic mechanism of weight loss from fresh fruit and vegetables is due to vapor-phase diffusion caused by difference in water vapor pressures at different location where the product is stored (Yaman and Bayoindirli, 2002) and besides that respiration also results in loss of weight of fruits as it involved degradation and loss of carbon atom from the fruit (Bhowmik and Pan, 1992).

The firmness of the fruits was significantly ( $p < 0.05$ ) reduced with storage time for both hot water treated and control fruits (Figure 1B). However, there was no significant difference observed between tomato fruits treated at 40 and 50°C for 20 min (Figure 1B). At the end of the storage period, control fruits had lower firmness values than that of the treated ones. On the other hand, the maximum firmness was maintained by treated fruits. Similar result is reported by (Tigist et al., 2012) which showed that the firmness of control fruit decreased more than that of treated fruits. Fruit softening results from cell structure deterioration and changes in composition of cellular material and cell wall (Seymour et al., 2002). This is a biochemical process involving pectin and starch hydrolysis by enzymes like wall hydrolases. Depolymerization (shortening of chain length of pectin substances) occurs with an increase in pectinesterase and polygalacturonase activities during fruit ripening (Yaman and Bayoindirli, 2002).

#### Effect of hot water treatment on reduction of chilling injury and extension of shelf life of tomato fruit

Chilling injury index was significantly ( $p < 0.05$ ) higher in control fruits than treated ones (Table 1). Fruits treated by

hot water showed the lowest outbreak of skin lesion than control. Chilling injury in tomato fruits was observed as susceptibility to skin lesion, and failure to ripe. These symptoms mainly occurred upon removal from chilling to a warm, non-chilling temperature. Therefore, the extent of CI could be measured as the subsequent increases in skin lesion after chilling and ripening. According to Manurakchinakorn et al. (2014) heat treatment that increases chilling tolerance is believed to work through the induced synthesis and accumulation of specific heat shocked proteins (HSPs). Beside, Li et al. (2003) suggested 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.

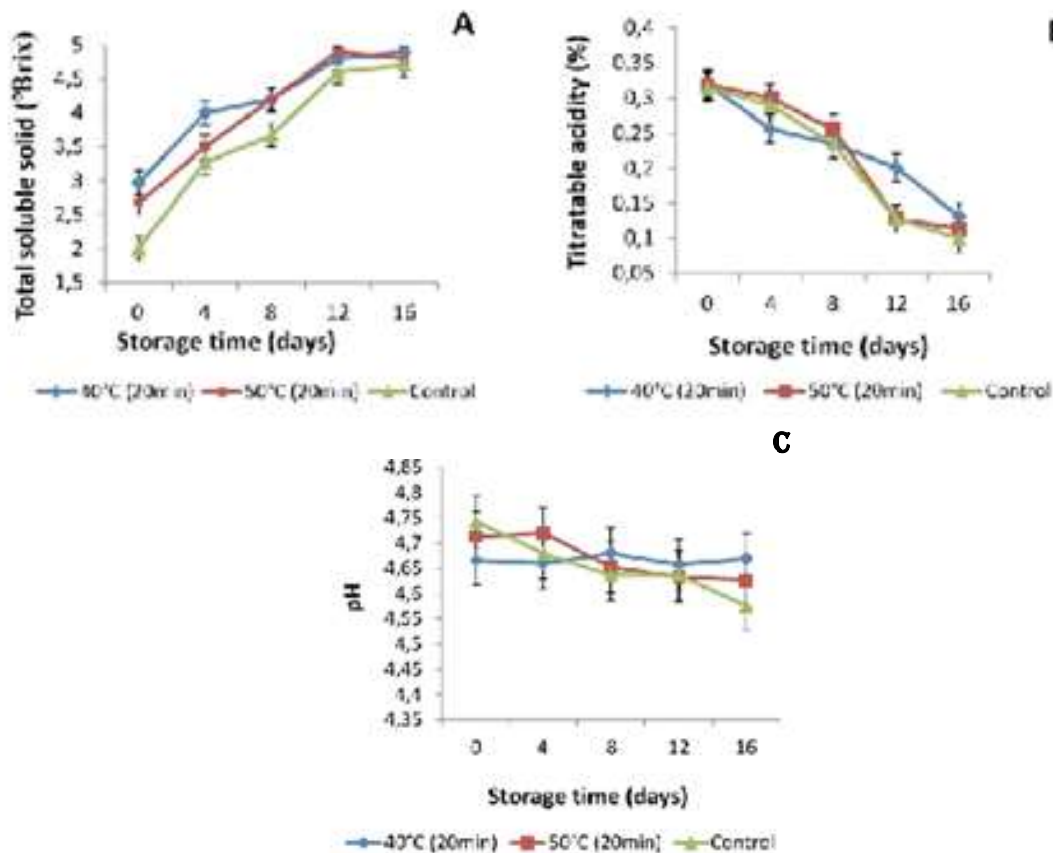
Shelf life of hot water treated fruits significantly ( $p < 0.05$ ) higher than control fruit (Table 1). This might be due to the valuable additional features of hot water treatment to enhance the quality of fruit result in shelf life extension and food safety.

#### Effect of hot water treatment on total soluble solid (TSS), titratable acidity (TA) and pH of the fruit

A statistically significant differences ( $p < 0.05$ ) was observed among the treatments in TSS. Higher TSS was recorded in hot water treated fruits than in control fruits (Figure 2A). This might be due to the fact that the extent of chilling injury was higher to cause skin lesion in fruits of control treatment. This in turn reduces the synthesis and utilization of metabolites resulting in lower TSS. It has been reported that fresh tomatoes showed more TSS than stored ones, with or without treatment (Kagan- Zur and Mizrahi, 1993). As shown in Figure 2A there was a

**Table 1.** Assessment of chilling injury and shelf life in tomato fruits stored at 4°C for two weeks (16 days) and subsequent ripening at 20°C for 16 days.

Treatment	Parameter	
	Chilling injury index	Shelf-life (day)
40°C (20 min)	0.193 <sup>a</sup>	17.33 <sup>bc</sup>
50°C (20 min)	0.299 <sup>b</sup>	16.67 <sup>b</sup>
Control	0.305 <sup>bc</sup>	14.00 <sup>a</sup>
CV (%)	15.0	5.1



**Figure 2.** Effect of hot water treatments on Total soluble (A), titratable acidity (B) and pH (C) of fruit during storage at 20°C. Control (—▲—), 40°C (20min) (—◆—) and 50°C (20min) (—■—). Data are means  $\pm$  SE.

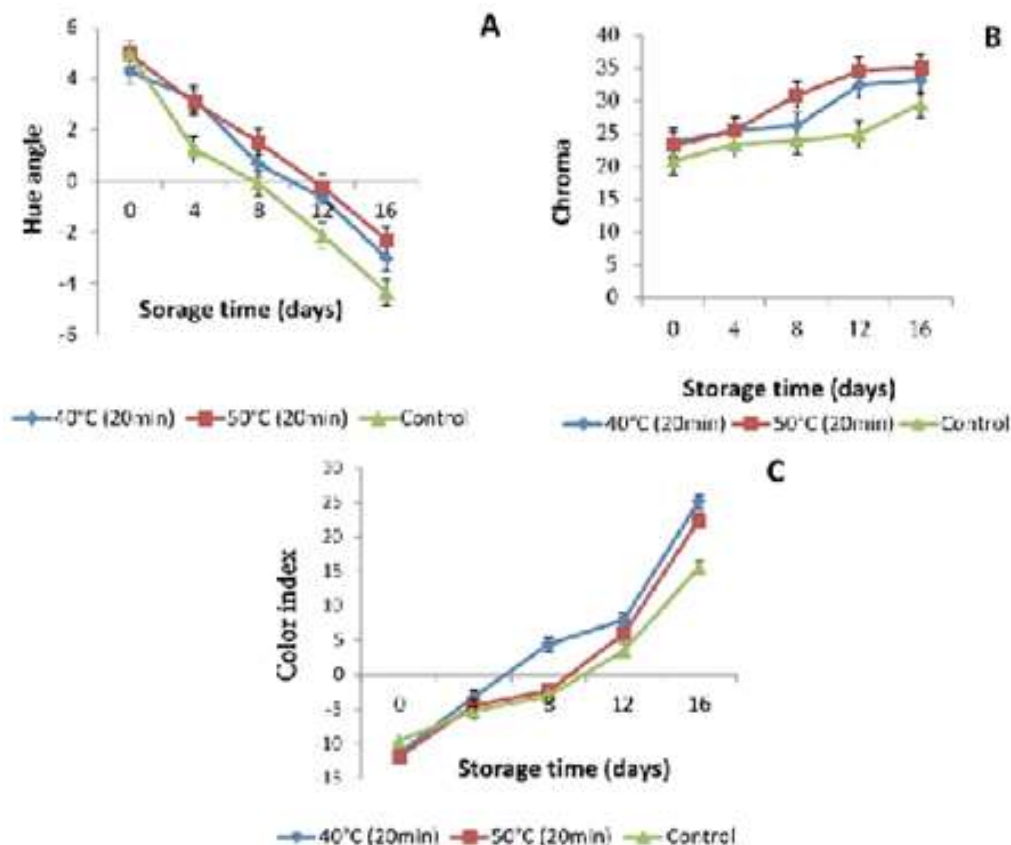
higher TSS in a treated tomato fruits during the gradual increase of TSS during the storage period.

There was no statistical difference observed among treated and control fruits in titratable acidity of the tomato fruits. However, the values for titratable acidity of treated and control fruit during storage decreased with storage time, with lower titratable acidity in the control treatment than fruits treated with hot water (Figure 2B). Organic acids, including malic or citric acid, are primary substrates for respiration phenomenon, therefore, a decrease of acidity is anticipated in highly respiring fruit (El-Anany et al., 2009). The pH of hot water treated and

control fruits fluctuated during the storage period (Figure 2C). However, at the end of the storage period, the differences between final pH values for all samples were not statistically significant.

#### Effect of hot water treatment on color of tomato fruit during storage

Figure 3 summarizes the effect of hot water on color of tomato during storage. The values for hue angle of tomatoes significantly ( $p < 0.05$ ) differed among the



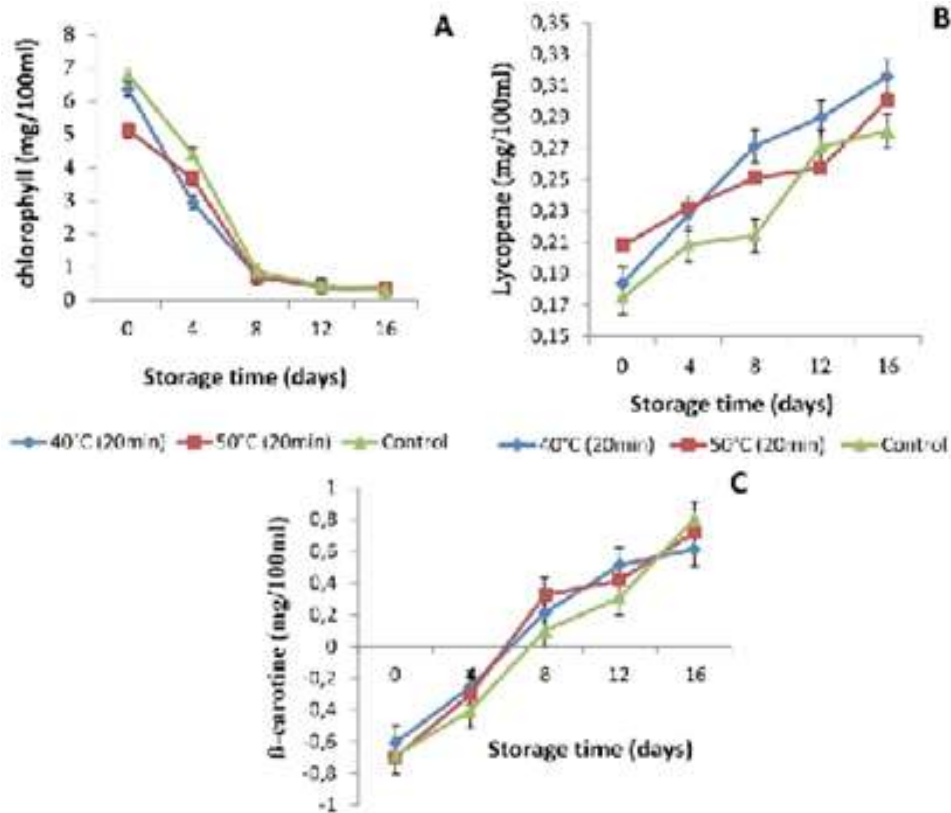
**Figure 3.** Effect of hot water treatments on hue angle (A), chroma (B) and total colour change (C) of the fruit during storage at 20°C. Control (—▲—), 40°C (20min)(—●—) and 50°C (20min)(—■—). Data are means  $\pm$  SE.

treatments, showing a decrease with the storage time for the control and hot water treated samples. This suggested that tomato fruits gained deep red color over storage time (Figure 3A). The rate of reduction in hue angle of fruits treated by hot water was low compared with fruits in control treatment. The result indicated that hot water treatment of the fruits can retain the color of tomato fruit. Both in hot water treated and control fruits, chroma was significantly ( $p < 0.05$ ) increasing during storage (Figure 3B). The rate of increment was significantly ( $p < 0.05$ ) higher in hot water treated fruits than in control ones. The final values of chroma in fruits treated at 50°C for 20 min increased during storage, showing the retention of redness in tomato fruits. The color index in the control fruits was significantly lower ( $p < 0.05$ ) than that of hot water treated fruit (Figure 3C). The significant ( $p < 0.05$ ) increment in color index might be an indication of the development of deep red color in tomato. The result indicated that hot water treatment of tomato fruits retained the redness of the fruit even after 16 days of storage. Color is one of the important quality attributes of tomato for consumer acceptability (Lim et al., 2010). Our study suggests that some negative changes

attributed to chilling injury during ripening and storage of the fruits at non-chilling temperatures may have taken place, affecting the visual quality parameters in untreated tomato fruits upon ripening and storage. Both McDonald et al. (1999) and Soto-Zamora et al. (2005) observed that hot water treatment increased respiration and ethylene evolution, and that red color development was enhanced by heat treatment and inhibited by chilling.

#### **Effect of hot water treatment on chlorophyll, $\beta$ -carotene and lycopene content of tomato fruit during storage**

Chlorophyll content of both treated and control green tomato fruits decreased during storage. The chlorophyll content in the control was higher than that of the hot water treated fruits (Figure 4A) until day eight. However, there was no difference among the treatments on their chlorophyll content of the fruits after day eight (Figure 4A). This implies that fruits without hot water treatment did show a degradation of chlorophyll, although they retained more chlorophyll than the treated fruits. In



**Figure 4.** Effect of hot water treatments on chlorophyll (A), lycopene (B) and beta-carotene (C) content of tomato fruit during storage at 20°C. Control (—●—), 40°C (20min)(—▲—) and 50°C (20min)(—■—). Data are means ± SE.

addition, fruits treated with 40 and 50°C for 20 min rapidly lost original chlorophyll after chilling and ripening (Figure 4A).

The accumulation rate of lycopene and β-carotene in the control fruits was slow compared to hot-treated tomato fruits (Figure 4 B, C). The value of lycopene synthesis of tomato fruits significantly ( $p < 0.05$ ) increased compared to control fruits while no difference was observed in β-carotene content among all the treatments. Although there was a significant decrease in chlorophyll content in the control (Figure 4A), it was not accompanied by the generation of lycopene and carotenoids. Chlorophyll degradation and lycopene accumulation, which are the most important processes during fruit ripening and senescence, commenced in treated tomatoes after transfer from 4 to 20°C. The generation of the normal red color in ripening fruits is the result of chlorophyll destruction and accumulation of carotenoids and lycopene. The present study showed treatment at 40 and 50°C for 20 min could maintain the capability of lycopene and carotenoids synthesis which would otherwise be interrupted by chilling stress. Moreover, the failure to ripe (accumulation of lycopene and carotenoids) in control fruits could be the result of

interruption in the conversion of chloroplasts to chromoplasts due to the destruction of plastids under the chilling temperature.

## Conclusion

From the current study, we could conclude that hot water treatment of tomato fruit at 40 and 50°C for 20 min could significantly maintain quality of tomato fruits by enhancing physical and quality attributes. In physical quality parameters, 40°C treatment for 20 min reduces weight loss, reduces chilling injury index and increases fruit firmness during storage. In addition, it increases shelf life better than control by three and half days on average. With regard to chemical quality attributes, 50°C treatment for 20 min is better for higher lycopene content compared to other treatments.

## Conflict of Interests

The authors have not declared any conflict of interests.

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