INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–476/2013/15–4–713–718
http://www.fspublishers.org

Full Length Article

Conditioning at Certain Temperature and Durations Induces Chilling Tolerance and Disease Resistance in Sweet Orange

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Abstract

Fruit of sweet orange (*Citrus sinensis* L.) was conditioned at 35, 40, 45 and 50°C for 12 or 24 h before storage at chilling temperature (2.5°C) for 75 days. The chilled fruits were evaluated after 75-day storage at 2.5±1°C either immediately or after 7 days of incubation period at ambient temperature (18–25°C) for chilling tolerance, disease resistance and quality of sweet orange fruits. The conditioning temperatures 40 and 45°C significantly reduced the weight losses, ion leakage, disease incidence and the lesion diameter but increased the surface pitting and declined the ascorbic acid. Temperature conditioning was more beneficial with 12 h duration where weight loss and lesion diameter were significantly lower while reducing sugars and ascorbic acid were significantly higher than 24 h. The post-chilling incubation resulted in significant increase in chilling induced damage.

Keywords: Sweet orange (*Citrus sinensis* L.); Conditioning temperatures; Post-chilling incubation durations; Chilling injury; Quality attributes

Introduction

Citrus sinensis L. is grown over an area of 199.940 thousand hectares with a production of 2132.276 thousand tons Pakistan (MINFAL, 2009). The supply of the citrus fruit is limited to the months of December to February and a considerable quantity is lost due to inadequate postharvest handling, transport, storage and marketing (Farooqi, 1994). The citrus fruits are chilling sensitive and hence storage at low temperature may cause chilling injury (Couey, 1986). Prolonged storage at low temperature can also be detrimental to citrus fruit quality due to its chilling sensitivity (Porat *et al.*, 2004), and quality of fruit is further lost when it is removed from chilling temperature (Saltveit, 1989). According to El-hilali *et al.* (2003) the storage of "Fortune" mandarin fruit at temperatures below 8°C resulted in severe peel pitting, characteristic symptom of chilling injury in citrus fruit, before even transferring to ambient temperature. The severity of chilling injury increased with decreasing temperature and increasing storage duration. Chilling injuries are characterized by surface pitting, mealiness, water soaked areas and enhanced loss of water, which in turn result in high levels of reducing sugars, ascorbic acid, anthocyanin and greater susceptibility to decay (Dugo *et al.*, 2003).

The chilling sensitivity and decay susceptibility have been the subject of research throughout the world. The focus of research is to decrease chilling sensitivity and induce tolerance to pathogens. Temperature conditioning (Wang, 1993; Farooq *et al.*, 2008; Tommasini), intermittent warming (Cabrera and Saltveit, 1990), heat shocks (Collins *et al.*, 1995), and chemical shocks (Jenning and Saltveit, 1994) are some of the measures that induce tolerance to chilling. Temperature conditioning refers to holding the fruit at certain low or high temperature before storage at low temperature and has been found to induce tolerance to chilling (Saltveit, 1991). Rahemi and Mirdehgan (2004) studied the effects of high temperature conditioning on reducing chilling injury in pomegranate fruits by exposing to 38°C for 0 (control), 12, 24 and 36 h before storage at 1.5°C and 85±5% relative humidity for 4.5 months and reported significant decrease in chilling injury symptoms (browning) and weight loss but had no significant effect on electrolyte leakage, total soluble solids, total acidity, ascorbic acid and pH of fruits after removal from the storage. A change in the concentration of carbohydrates, especially sucrose is also involved in chilling tolerance in citrus fruit (Murata, 1994; Farooq *et al.*, 2009). Holland *et al.* (2005) reported that temperature conditioning induced alterations in carbohydrate metabolism occurring during the postharvest storage of citrus fruit which favored sucrose, but not hexoses, accumulation and its maintenance after the fruit was transferred to low temperature.

The present study was, therefore, initiated to investigate the influence of conditioning temperature and duration on chilling sensitivity of sweet orange fruit.

Materials and Methods

The sweet orange fruit was exposed to temperature conditioning (T) at 35, 40, 45 and 50°C for 12 or 24 h (D)

To cite this paper: Sajid, M., A. Rab, I. Jan, I. Haq and M. Zamin, 2013. Conditioning at certain temperature and durations induces chilling tolerance and disease resistance in sweet orange. *Int. J. Agric. Biol*., 15: 713–718

before exposure to chilling storage at 2.5°C for 75 days. The conditioned fruit in each treatment after the chilling were analyzed for different parameters immediately after the chilling exposure and after 7 days incubation (IT) at 20°C as simulated marketing time. The rate of weight loss was determined by measuring weight loss with the help of an electronic scale immediately after the chilling and after 7 days simulated marketing time. The same fruits were also used for determining other parameters such as surface pitting. Reducing sugars and ascorbic acid were determined according to AOAC (1990). Disease incidence and expansion were evaluated by inoculating 10 fruits for each treatment and replication, with culture of *Penicillium itallicum* and *P. digitatum* for 24 h before administering the heat treatments. *P. italicum* and *P. digitatum* were grown on PDA solution in Petri dishes at 25ºC for 7 to 10 days. Spores were rubbed from the agar surface and a high-density spore suspension was prepared. Oranges were inoculated 1 mm deep into the flesh in the equator of two opposite faces with a plastic syringe. Approximately 0.25 mL of the spore suspension was applied at each inoculation point. The ion leakage was determined by taking fruit rind discs of 5 mm diameter from the equatorial region of the fruit. The rind discs were then placed in Petri-dishes over wet paper towels. The ion leakage from the rind discs was recorded immediately after 75 days exposure to chilling temperature (2.5°C), or after 7 days incubation at room temperature. Data were recorded by shaking the discs in 0.5 M mannitol solution for 30 and 90 min on a rotary shaker. The initial conductivity 30 min are regarded as ion leakage from cell wall, while the conductivity reading after 90 minutes indicate leakage from cytoplasm due to changes in cell membrane (Saltveit, 1989). The total conductivity was recorded after 3 cycles of freezing and thawing. Ion leakage from the cytoplasmic component is presented as percent of total ion leakage from the tissue.

The experiment was run in a completely randomized design (CRD) with factorial arrangement. Comparison between means was evaluated by Duncan's multiple range test at 5% level of significance. All storage treatments were done with three replications.

Results and Discussion

Rate of Weight Loss

The rate of weight loss day^{-1} was significantly affected by conditioning temperatures (T). The rate of weight loss day^{-1} was 1.12%with fruits conditioned at 35°C and chilled at 2.5°C for 75 days. Weight loss decreased, though non significantly, to 0.65 and 0.80% day^{-1} with conditioning at 40 and 45°C, respectively but increased again to the maximum of 1.09 % day^{-1} when fruit were conditioned at 50°C (Table I). The conditioning duration (D) effect was also significant. The weight loss day^{-1} was significantly lower (0.86% day^{-1}) with temperature conditioning for 12 h as compared to 2.66%, when conditioning duration was extended to 24 h. The post-chilling incubation time (IT) at ambient temperature also significantly affected the weight loss in sweet orange fruit. The weight loss increased significantly from 0.25% day^{-1} to 3.27% day^{-1}. The interaction between conditioning temperature and post-chilling incubation (T × IT) was also significant (Fig. 1). The rate of weight loss day^{-1} was the lowest (0.16% day^{-1}) when fruits were conditioned at 40°C and analyzed immediately after the chilling exposure as compared to the maximum weight loss (8.57% day^{-1}) observed with conditioning at 50°C and after 7 days incubation at room temperature (Fig. 1). The fruit continued to lose water after harvest (Al-Obeed and Harhash, 2006), which is enhanced by chilling injury (Purvis, 1984) but temperature conditioning at 40 and 45° decreased the rate of water loss which increased with increasing conditioning duration at each temperature. Since water loss is a biological activity, it increased with increase in temperature (Kader, 1992). The significant increase in water loss after chilling exposure (2.5°C) is an indication that chilling injury in sweet orange fruit enhanced water loss (Cohen *et al.*, 1994). It has been reported that heat treatments at relatively high temperature increase weight loss in fruits (Erkan *et al.*, 2005) but modest conditioning temperatures (40–45°C) inhibited weight loss. According to Vicente *et al.* (2002) the initial increase in weight loss in heat-treated fruits decreased later at 20°C.

Surface Pitting

The temperature conditioning and post-chilling incubation time at ambient temperature (25 ± 2^{o}C with 60–70% relative humidity) significantly affected the surface pitting of sweet orange. Conditioning durations had a non-significant influence on surface pitting. Though non-significant, but surface pitting increased from 14.66 to 14.70% with increasing the conditioning temperature from 35 to 40°C, and grew to significant levels of 18.90 and 24.94% with increasing conditioning temperature to 45 and 50°C, respectively. The conditioning duration effect on surface pitting was non-significant but the post-chilling incubation at ambient temperature significantly affected the surface pitting on sweet orange fruit. The surface pitting increased significantly from 14.47% on day 0 (immediately after 75 days chilling at 2.5°C) to 22.13% when the fruit were evaluated after 7 days incubation at ambient temperature (Table I). The interaction between conditioning temperature and post-chilling incubation time significantly influenced the surface pitting (Fig. 2). The maximum surface pitting 31% was recorded with 50°C and 7 days post-chilling incubation at ambient temperature as compared to the minimum (12.1%) observed with conditioning at 40°C and 0 days post-chilling incubation (Fig. 2). Surface pitting is a peel disorder of citrus fruits (Petracek *et al.*, 1995) caused by abiotic stresses as water stress, chilling injury or heat treatments that lead to phospho-lipid catabolism and cellular

Table 1: Effect of conditioning temperature and durations, and post-chilling incubation on fruit quality, chilling sensitivity and disease susceptibility of sweet orange fruit

Treatments	Weight loss (%)	Surface pitting (%)	Ion leakage (%)	Disease incidence (%)	Lesion diameter (mm)	Reducing sugar (%)	Ascorbic acid (mg ml^{-1})
Conditioning temperature (°C)							
35	1.12 a	14.66 c	22.53 b	18.33 a	10.25 a	4.65 b	34.10 a
40	0.65 b	14.70 c	20.88 b	12.00 b	5.27 b	5.24 a	27.55 b
45	0.80 ab	18.90 b	22.72 b	11.00 b	4.76 b	5.29 a	20.47 c
50	1.09 a	24.94 a	26.96 a	13.58 b	8.71 a	4.79 b	14.03 d
LSD at α 0.05	0.1306	3.261	3.626	3.584	1.957	0.424	4.269
Conditioning duration (h)							
12	0.86	17.23	22.37	13.21	6.34	5.15	25.81
24	2.66	19.36	24.17	14.25	8.15	4.83	22.25
Significance	*	ns	ns	ns	*	*	*
Post-chilling incubation time at ambient temperature (Days)							
0	0.25	14.47	20.24	6.29	1.58	5.28	29.47
7	3.27	22.13	26.30	21.17	12.92	4.70	18.59
Significance	*	*	*	*	*	ns	*
Interactions	Significance level						
D × T	* Fig1	*Fig2	* Fig3	* Fig 4	* Fig 4	---	---
T × IT	* Fig 1	---	---	---	---	---	---

Mean followed by similar letter(s) in column do not differ significantly from one another
ns= Non Significant and * = Significant at 5% level of probability
D × T = Interaction of storage duration and storage temperatures
T × IT = Interaction of storage temperatures and Incubation time

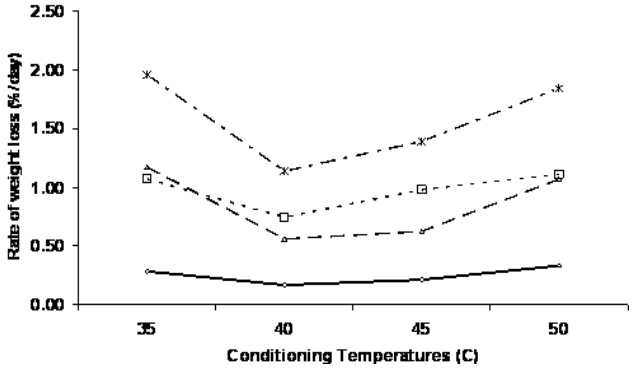

Fig. 1: Effect of conditioning temperatures and durations on the rate of weight loss after chilling (75 days at 2.5^{0}C) or post-chilling incubation at ambient temperature

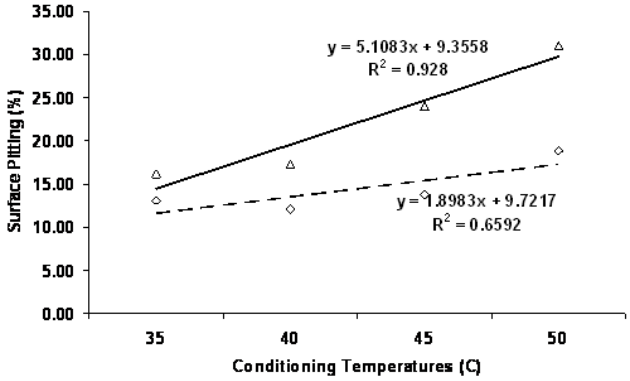

Fig. 2: Surface pitting (%) in sweet orange fruit after 75 days chilling at 2.5^{0}C or after 7 days incubation at ambient temperature

membrane breakdown (Liberman, 1958). Mao *et al.* (2007) have correlated surface pitting in cucumber fruit with membrane deterioration. The data related to the surface pitting indicated that surface pitting increased with increasing conditioning temperature or conditioning duration at each temperature or during post-chilling incubation at ambient temperature. Surface pitting is promoted both by chilling and high temperature (Arpaia and Kader, 2009). The chilling induced surface pitting is characterized by dark peel collapse, which form pits (Ritenour *et al.,* 2003).

Ion Leakage

The percent ion leakage was significantly influenced by conditioning temperature and post-chilling time at ambient temperature (25±2^{o}C with 60–70% RH) but conditioning durations showed a non-significant effect on percent ion leakage (Table 1). The interaction of conditioning temperature × conditioning duration had significant influence ion leakage of sweet orange fruit (Fig. 3). Ion leakage increased non-significantly from 20.88% to 22.72% with increasing conditioning temperature from 35 to 45°C but significantly to 26.96 with 50°C conditioning temperature. The conditioning duration effect was non-significant but post-chilling incubation time affected the ion leakage significantly. Ion leakage increased from 20.24% on day 0 (immediately after 75 days chilling at 2.5°C) to 26.30% after 7 days incubation at ambient temperature. Chilling injury is highly correlated with increased electrolyte leakage of skin tissue (Mao *et al.,* 2007) have co-related surface pitting in cucumber fruit with membrane deterioration, as determined by increased electrolyte leakage due to chilling. Heat treatments and temperature conditioning reduced chilling injury in variety of different tissue (Viachonasios *et al.,* 2001) thus, it is likely to observe

low ion leakage with modest conditioning temperatures (40–45°C). A non-significant difference was observed for the ion leakage from the peel of control and heat-treated banana fruit. However, significant increase in ion leakage was observed when conditioning was carried out at 50°C, which may be due damage to the membrane system in the tissue (Norma *et al.*, 1998).

Disease Incidence

The conditioning temperatures and post-chilling time at ambient temperature (25±2°C with 60–70% RH) had significant effect on disease incidence (Table 1). The conditioning durations had non-significant influence on disease incidence. The interaction of conditioning temperature and post-chilling time (D × T) at ambient temperatures (0 and 7 days) showed significant effect, but rest of the interactions were non-significant. Disease incidence decreased significantly with increasing the conditioning temperature, but a non-significant difference was noted when the conditioning temperature increased from 40 to 50°C. A significant decrease was observed in disease incidence from 18.33% at 35°C to 13.58% at 40–50°C. The disease incidence was non-significant for conditioning durations (12 and 24 h) but the post-chilling incubation time (0 and 7 days) at ambient temperature significantly affected the disease incidence of sweet orange fruit. The disease incidence increased significantly from 6.29% on day 0 (immediately after 75 days chilling at 2.5°C) to 21.17% when the fruit were evaluated after 7 days incubation at ambient temperature (Table 1). The interaction of conditioning temperature and post-chilling incubation time indicated that the disease incidence increased with increasing the post-chilling incubation time at ambient temperature (Fig. 4). The maximum disease incidence (24.33%) was recorded in fruits treated with 50°C conditioning temperature and analyzed after 7 days incubation at ambient temperature (Fig. 4). The sweet orange fruit are affected by several pathogens but *P. italicaum* and *P. digitatum*arethe are the most abundant pathogens (Plaza *et al.*, 2003), which may attack the fruit either before or after harvest (Snowdon, 1990). The decay causing organisms (fungi) are also favored by prolong chilling injury (Couey, 1986). The heat treatments have been shown to decrease the disease incidence. Ritenour *et al.* (2004) have reported that spores of *P. digitatum* are inactivated when exposed to 43.5°C forced vapor heat treatment for 270 min but the disease control depends on the moisture content and metabolic activity of the spores, exposure temperature and duration (Barki-Golan and Philips, 1991). Temperature conditioning is used to prevent chilling injury (Nair and Singh, 2009) and suppress disease incidence (Porat *et al.*, 2004). High temperature enhances wound healing (Schirra and Cohen, 1999) and promotes the synthesis of anti-fungal compounds (Kim *et al.*, 1991). Heat treatments also trigger the synthesis of heat shock proteins

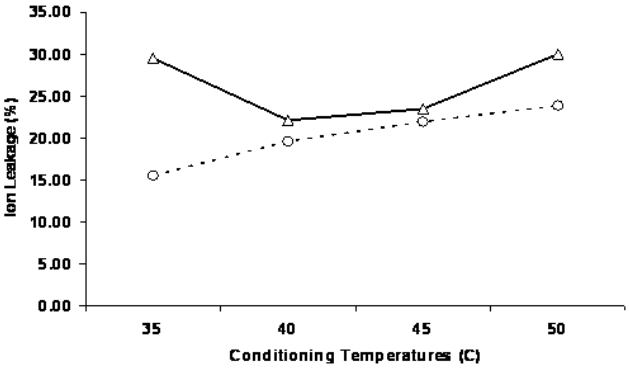

Fig. 3: Conditioning temperature and duration affect the ion leakage from rind of sweet orange fruit

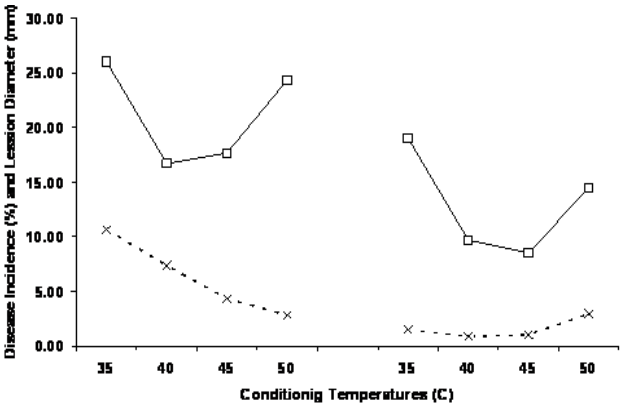

Fig. 4: Effect of conditioning temperature and chilling on disease incidence and lesion diameter (mm) of sweet orange fruit

(Saltveit *et al.*, 2004), which contribute to the acquisition of thermo-tolerance (Vierling, 1991). These results confirm the effectiveness of high temperature conditioning (40–50°C) for disease suppression in citrus fruits, and temperatures below the stated temperature (40–45°C) may not suppress pathogens on sweet orange fruits (Ritenour *et al.*, 2003). The non-significant effect of conditioning duration indicated that do not induce any harmful effect on sweet orange fruits. However, the significant increase in disease incidence during post-chilling incubation demonstrate that 75 days chilling at 2.5°C caused sufficient and irreparable injury to the fruits (Rab and Saltveit, 1996) enhanced by susceptibility to pathogens (Saltveit, 1991).

Disease Expansion (Lesion Diameter)

The conditioning temperatures, durations and post-chilling incubation time at ambient temperature (25±2°C with 60–70% RH), interaction of conditioning temperature and post-chilling time at ambient temperatures (days) had significant difference for lesion diameter, but all other interactions were non-significant (Table 1). The lesion diameter significantly decreased with increasing the

conditioning temperatures. But the conditioning temperatures of 35 and 50°C were non-significant. Similarly a non-significant response was observed for the conditioning temperature of 40 and 45°C having lesion diameter of 5.27 mm and 4.76 mm. The conditioning durations significantly increased the lesion diameter from 6.34 mm to 8.15 mm at 12 and 24 h, respectively. The lesion diameter increased from 1.58 mm to 12.92 mm at post-chilling time at ambient temperature from 0 to 7 days. The interaction of conditioning temperature and post-chilling incubation time significantly affected the lesion diameter (Table 1). The maximum lesion diameter (19.0 mm) was noted at post-chilling incubation time of 7 days with 35°C conditioning temperature as against the minimum lesion diameter (0.875 mm) recorded immediately after chilling at conditioning temperature of 40°C. The decrease in lesion diameter with increasing conditioning temperature (35–45°C) indicated that temperature conditioning decreased disease incidence (Ritenour *et al.,* 2004) by accelerating the wound healing of fruit and reducing the green mold decay caused by *P. digitatum* (Erkan *et al.,* 2005). Thus, temperature conditioning at 40 or 45°C decreased disease incidence and expansion (Porat *et al.,* 2004) but greater lesion expansion at 50°C indicated that very high temperature may damage the tissue and increase its susceptibility to decay. The control of disease by heat depends on the moisture content, metabolic activity of the spores, exposure temperature and durations (Ritenour *et al.,* 2003). It seems as if some of the spores might have escaped the temperature induced killing, and result in increased disease incidence due to increasing cuticular cracks (Joyce *et al.,* 2003) with conditioning at 50°C. It may explain the increased disease lesion diameter with increasing conditioning duration or after 7 days post-chilling incubation at ambient temperature.

Reducing Sugar

The conditioning temperature and conditioning durations had significant effect on reducing sugar (Table 1). The post-chilling incubation time at ambient temperature (0 and 7 days) had non-significant effect on reducing sugar. A significant variation in reducing sugars was recorded with increasing the conditioning temperatures from 35 to 50°C. The conditioning temperatures of 40 and 45°C showed non-significant response for reducing sugar. Similarly, a non-significant difference was recorded with conditioning temperature of 35 and 50°C for reducing sugar. The reducing sugars decreased significantly with increasing the conditioning durations from 12 to 24 h. The maximum reducing sugar (5.15%) was recorded with 12 h conditioning duration as compared to 24 h (4.83%). According to Holland *et al.* (1999) the reducing sugars increased during chilling but the post-chilling enhanced respiration caused rapid decline of reducing sugars in chilled fruits (Ribas-Carbo *et al.,* 2000). Thus, the significantly lower reducing sugars could be due to chilling injury to citrus fruits (Nair *et al.,* 2001) but decline of reducing sugars at 50°C cannot be explained by normal respiratory metabolic depletion. It is possible that exposure to 50°C may invoke fermentative metabolism leading to significant decline in reducing sugars (Echeverria and Ismail, 1987).

Ascorbic Acid

Conditioning temperatures, conditioning durations and post-chilling incubation time at ambient temperature (days) had significant variation for ascorbic acid but all of their interactions were non-significant (Table I). The ascorbic acid of sweet orange significantly decreased with increasing the conditioning temperatures from 35 to 50°C. The maximum ascorbic acid (34.10 mg mL^{-1}) was recorded in fruits conditioned at 35°C as compared to 14.03 mg mL^{-1} observed for 50°C. A significant difference was also recorded for conditioning duration. The maximum ascorbic acid (25.81 mg mL^{-1}) was noted in fruits conditioned for 12 h as compared to fruits conditioned for 24 h (22.25 mg mL^{-1}). The post-chilling incubation time at ambient temperature significantly reduced the ascorbic acid after 7 days (18.59 mg mL^{-1}) in contrast to 0 day (29.47 mg mL^{-1}). The decrease of ascorbic acid with storage is a common phenomenon (Abbasi *et al.,* 2009). Ascorbic acid was maximum in fruits conditioned at 35°C, which declined with increase in conditioning temperature or duration or post-chilling incubation. Earlier studies suggested that high temperature may result in low decline of ascorbic acid (Antonio *et al.,* 2004) or low (Erkan *et al.,* 2005) during chilling. However, our findings suggested that ascorbic acid declined both as function of temperature and duration. Higher the conditioning temperature, longer the conditioning duration or post-chilling incubation, the greater was the loss of ascorbic acid in sweet orange fruit.

Conclusion

Chilling increased the weight loss, incidence of surface pitting, loss of reducing sugars and ascorbic acid, disease incidence and susceptibility and ion leakage from the flavedo tissue. Temperature conditioning at 40 or 45°C for 12 h was the most effective technique to suppress such losses in fruit quality. An extended conditioning duration (24 h) promoted such quality damage of fruit. Thus, while temperature conditioning at 40 or 45°C for 12 h may offer a good procedure for inducing chilling tolerance, but it increased the loss of ascorbic acid.

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(Received 12 July 2012; Accepted 17 November 2012)