Effects of Intermittent Warming and Temperature Conditioning on the Postharvest Quality of 'Oroblanco' Citrus Fruit Following Long-term Cold Storage

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SUMMARY. **'Oroblanco' is an early-maturing pummelo-grapefruit hybrid (*Citrus grandis* x *C. paradisi*). The fruit of this cultivar are usually picked in October and are marketed while their peel color is still green. However, during long-term storage, the fruit turns yellow, and loses much of their commercial value. In a previous study, we found that application of gibberellic acid and low storage temperatures of 2 ºC (35.6 ºF) markedly reduced the rate of degreening. However, 'Oroblanco' fruit are sensitive to chilling injuries, and thus could not be stored at 2 ºC for long periods. In the present study, we examined the possible application of intermittent warming (IW) and temperature conditioning (TC) treatments, in order to retain the green fruit color during long-term cold storage but without enhancing the development of chilling injuries. It was found, that following storage at 2 ºC, either with or without IW and TC, the fruit retained green color up to 16 weeks, whereas at 11 ºC (51.8 ºF) fruit turned yellow after 8 weeks. However, untreated fruit held continuously at 2 ºC developed 40, 51, and 68% chilling injuries after 8, 12, and 16 weeks, respectively. IW (storage at cycles of 3 weeks at 2 ºC + 1 week at 11 ºC) reduced the amount of chilling injuries to only 5, 7 and 11% after the same periods of time, respectively. TC [a pre-storage treatment for 7 days at 16 ºC (60.8 ºF) before continuous storage at 2 ºC] effectively reduced the development of chilling injuries to only 5% after 8 weeks of storage, but was ineffective in reducing chilling damage after longer storage periods. Because chilling damaged fruit is prone to decay, the IW and TC treatments also reduced the incidence of decay development during storage. The IW and TC treatments did not affect juice total soluble solids and acid percentages, but did affect fruit taste and the amounts of off-flavor volatiles emitted from the juice. Taste panels indicated that the taste score of untreated control fruit stored at 11 ºC gradually decreased during long-term storage, and that this decrease was more severe in chilling damaged fruit stored continuously at 2 ºC. The taste of IW-treated fruit remained acceptable even after 16 weeks of storage, and TC-treated fruit remained acceptable for up to 12 weeks. Fruit taste scores were inversely correlated with the concentrations of ethanol and acetaldehyde detected in the juice headspace.**

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Oroblanco is a triploid pummelo-grapefruit hybrid that was generated by a cross between an acidless pummelo and a tetraploid grapefruit (Soost and Cameron, 1981). The general characteristics of the fruit are similar to those of 'Marsh Seedless' white grapefruit, despite the fact that 'Oroblanco' has a higher percentage of total soluble solids (TSS), a lower acid content, and a special sweet aroma that is more similar to pummelo (Soost and Cameron, 1981).

'Oroblanco' fruit are early maturing, and according to their internal characteristics are ready for eating in October, when the peel color is still green (Soost and Cameron, 1981; Traiber et al., 1984). Commercially, the fruit are picked during a short period of time between October and November, and are exported and marketed when their color is still green. Unfortunately, during postharvest storage the fruit tends to turn yellow, and as a result loses much of its commercial value.

In a previous study, we found that application of gibberellic acid and low storage temperatures are critical for maintaining the fruit's green color (Porat et al., 2001). In fact, gibberellic acid sprays are currently being applied to the fruit as part of the horticultural practice given in the orchard (Greenberg et al., 1986). However, just as its grapefruit and pummelo parents, 'Oroblanco' fruit are sensitive to chilling injuries, and it is currently impossible to store the fruit at low temperatures for long periods (Chalutz et al., 1985; Waks et al., 1988). The chilling injuries in 'Oroblanco' fruit are usually observed as pitting throughout the peel surface or as 'watery breakdown'. In the current study, we evaluated the effects of possible applications of intermittent warming (IW) and temperature conditioning (TC) techniques, in order to store the fruit for long periods of time at low temperatures and to retain their green color without enhancing the development of chilling damage.

IW is a practical commercial practice that involves periodic interruptions of the cold storage period by exposure of the fruit to short warming periods (Wang, 1993; Cohen, 1999). It was reported that IW, using various warm and low temperature combinations, effectively reduced the development of chilling injuries in oranges (C. sinensis), grapefruit, mandarins (*C. reticulata*), and lemons (*C. limon*) (Cohen, 1988; Cohen et al., 1983; Davis and Hofmann, 1973; Schirra and Cohen, 1999; Schirra and Mulas, 1995). In lemons, warming the fruit for 7 d at 13 °C (55.4 °F) following every 21 d of cold storage at 2 °C eliminated the development of chilling injuries and retained market quality for a period of up to 6 months (Cohen et al., 1983; Cohen, 1988). In the current study, we chose to evaluate the effects of this same IW procedure, in order to extend the postharvest storage and marketing period of 'Oroblanco' fruit.

Prestorage TC is a commercial practice in which the fruit are held in either low- or high-temperatures for short periods of time before continuous storage at low temperatures (Hatton, 1990; Wang, 1993). In citrus, it was reported that the development of chilling injuries could be reduced by conditioning the fruit for 7 d at 16 °C before cold storage (Hatton and Cubbedge, 1982, 1983; Porat et al., 2000). In the current study, we further evaluated the effects of this conditioning treatment on reducing chilling damage during long-term cold storage of 'Oroblanco' fruit.

Materials and methods

Plant material and storage conditions. 'Oroblanco' fruit were purchased from a commercial packinghouse, in which the fruit were washed and waxed with a carnauba-based coating containing 2 $g \cdot L^{-1}$ (2,000 ppm) imazalil (SafePack Ltd., Cfar Saba, Israel). In all experiments, the fruit were picked between middle to late October, and were used the day after harvest. For postharvest evaluations, fruit were stored for 8, 12, or 16 weeks at 2 °C followed by an additional week of shelf-life conditions at 21 °C (69.8 °F). The relative humidity in the storage rooms was about 85%. Each treatment included four boxes, each containing 25 fruit (100 fruit per treatment). The experiments were repeated two times (once each season) during two consecutive seasons.

IW and TC treatments. IW was applied by placing the fruit for 3 weeks in a storage room at 2 °C and afterwards transferring the fruit for another week to another storage room at 11 °C. The 8-week storage period consisted two such IW cycles; the 12-week storage period consisted three such IW cycles; and the 16-week storage period consisted four IW cycles.

The TC treatment was performed by pre-storing the fruit for 7 d at 16 °C, and afterwards transferring them to continuous storage at 2 °C.

Color measurement. Twenty green fruit per treatment were circled with a black marker, and the hue angle of the peel within these circles was determined at the beginning and at the end of each storage period with a chromameter (model CR-200; Minolta, Osaka, Japan), as described by McGuire (1992). A hue angle of 120° represents green color and 100° is equal to yellow.

Assessment of chilling injury, decay, and weight loss. At the end of each storage period, the fruit were evaluated, and the percentages of chilling injured and decayed fruit were calculated according to the initial amount of fruit examined. In addition, 15 fruit per treatment were weighed before and after storage to assess the percentage of weight loss.

Chemical analysis. The TSS in the juice of 10 individual fruit was determined with a refractometer, and the percentage of acidity was measured by titration to pH 8.3 with 0.1 N sodium hydroxide. Ethanol and acetaldehyde levels were determined in the headspace of 50-mL erlenmeyer flasks containing 10 mL of juice aliquots incubated at 30 °C (86.0 °F) for 30 min. Afterwards, a 1-mL sample was withdrawn from the headspace and was analyzed by a gas chromatograph. The given ethanol and acetaldehyde levels are means of four replications per treatment, each consisting juice from three different fruit.

Taste score. Initially and at the end of each storage period, the same group of 15 untrained panelists tasted the fruit. Taste was scored on a scale of 0 to 10, where 0 = objectionable taste and 10 = excellent taste. The fruit were served as cut segments collected from 10 different fruit per treatment.

Statistical analysis. Student-Newman-Keuls one-way analysis of variance (ANOVA) tests on ranks were performed using the SigmaStat statistical software (Jandel Scientific Software, San Rafael, Calif.). In addition, regression analysis according to the General Linear Model (GLM) procedure was performed using the SAS statistical analysis program (SAS Institute Inc., Cary, N.C.).

Results

Effects of IW and TC on fruit degreening during long-term storage. Following storage at a low temperature of 2 °C, either with or without IW or TC treatments, there was only a slight change in the color of 'Oroblanco' fruit, whereas after storage at 11 °C, there was a progressive increase in the rate of degreening (Fig. 1). The average hue angle values at the beginning of the experiment and following 8, 12, and 16 weeks of storage at 2 °C were 121, 118, 116, and 112°, respectively (Fig. 1). On the other hand, the hue angle values following 8, 12, and 16 weeks of storage at 11 °C were 108, 102, and 95°, respectively (Fig. 1). A hue angle below 110° is considered unacceptable for marketing.

According to a regression analysis of fruit color across time for each temperature storage regime, it is concluded that the various 2 °C, IW and TC treatments were all significantly different at $P \leq 0.01$ from the continuous 11 °C treatment, but were not significantly different among themselves.

Effects of IW and TC on the

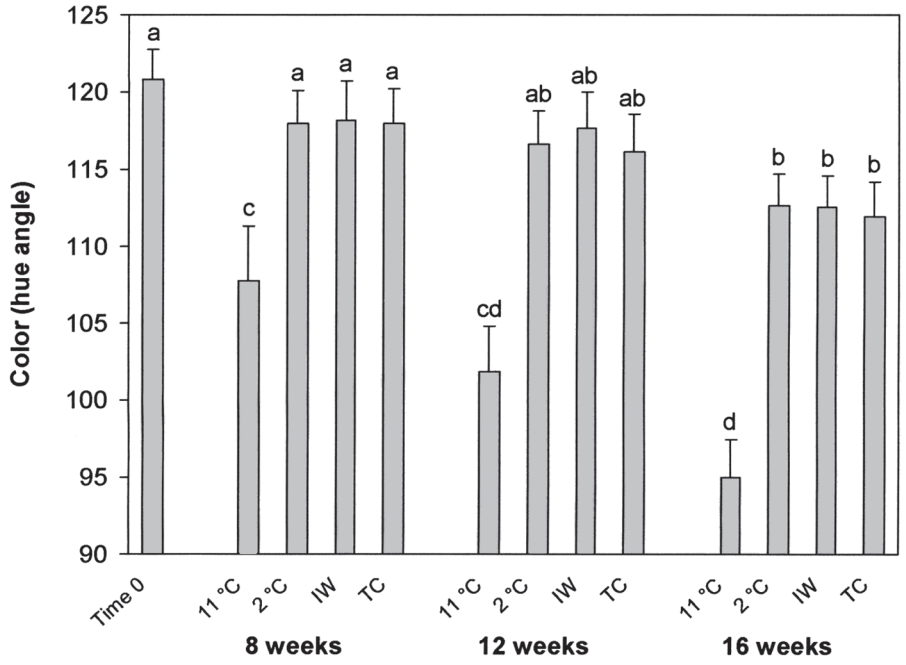

Fig. 1. Effects of storage temperatures, intermittent warming (IW), and temperature conditioning (TC) on the degreening of 'Oroblanco' citrus fruit. Fruit were stored at 11 °C (51.8 °F), 2 °C (35.6 °F), or handled under IW (storage at cycles of 3 weeks at 2 °C + 1 week at 11 °C) or TC [a prestorage treatment for 7 d at 16 °C (60.8 °F) before continuous storage at 2 °C] regimes. The hue angle of the peel was determined after 8, 12, and 16 weeks. Data are means ± SE of 20 fruit per treatment. Columns marked by different letters are significantly different at $P < 0.05$ according to a Student-Newman-Keuls one way analysis of variance test on ranks. A hue angle of 120° = green and 100° = yellow.

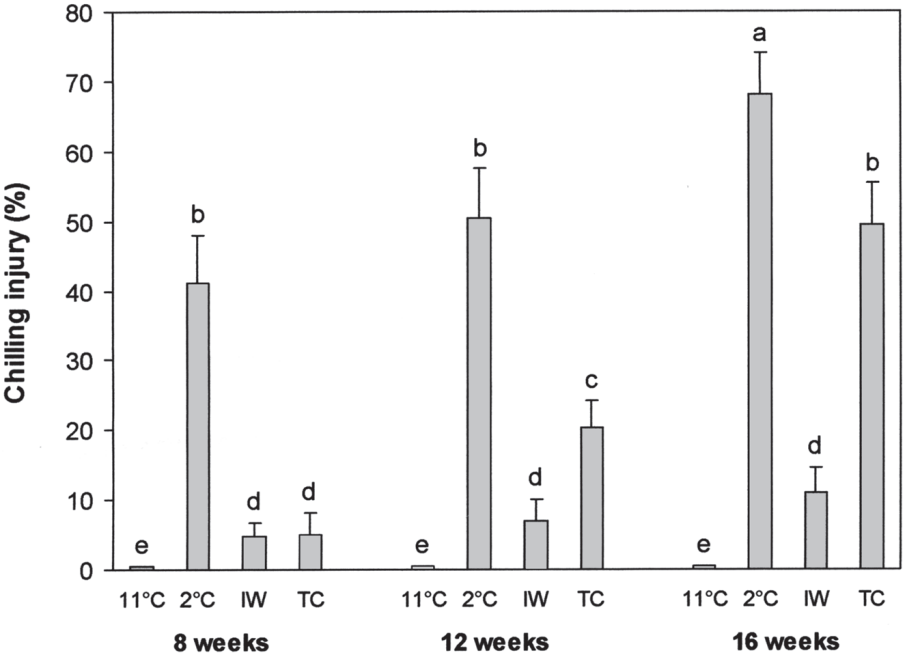

Fig. 2. Effects of storage temperatures, intermittent warming (IW), and temperature conditioning (TC) on the development of chilling injuries in 'Oroblanco' citrus fruit. Fruit were stored at 11 °C (51.8 °F), 2 °C (35.6 °F), or handled under IW warming (storage at cycles of 3 weeks at 2 °C + 1 week at 11 °C) or TC [a prestorage treatment for 7 d at 16 °C (60.8 °F) before continuous storage at 2 °C] regimes. The percentages of chilling injuries were determined after 8, 12, and 16 weeks. Data are means ± SE of 100 fruit per treatment. Columns marked by different letters are significantly different at $P < 0.05$ according to a Student-Newman-Keuls one way analysis of variance test on ranks.

DEVELOPMENT OF CHILLING INJURIES FOLLOWING LONG-TERM COLD STORAGE. Fruit stored continuously at 2 °C retained their green color, but 40%, 51%, and 68% chilling injuries developed following 8, 12, and 16 weeks of storage, respectively (Fig. 2). IW markedly reduced the development of chilling injuries throughout the entire storage period to only 5%, 7%, and 11% after 8, 12, and 16 weeks, respectively (Fig. 2). The TC treatment effectively reduced the amount of chilling injuries to only 5% after 8 weeks, but could not eliminate their appearance after longer storage periods (Fig. 2). Control fruit stored at 11 °C did not develop any chilling symptoms.

EFFECTS OF IW AND TC ON FRUIT QUALITY FOLLOWING LONG-TERM STORAGE. Control fruit stored at 11 °C and IW-treated fruit developed only negligible amounts of decay, whereas a high incidence of decay (16%, 21%, and 49%) developed following 8, 12, and 16 weeks, respectively, among the chilling-injured fruit stored continuously at 2 °C (Table 1). In TC-treated fruit, a massive amount of decay (23%) developed after 16 weeks, at which time the fruit had severe chilling injury (Table 1, Fig. 2).

After 8 and 12 weeks of storage, the rate of weight loss was more or less similar among the different treatments. However, after 16 weeks, the fruit stored at 11 °C lost significantly more weight as compared with the various other treatments stored at 2 °C (Table 1).

When evaluating juice characteristics, we could not detect and significant effects of the IW and TC treatments on juice TSS and acid percentages as compared with control fruit stored at 11 °C (Table 1). Nevertheless, the stress conditions conducted from continuous storage at 2 °C has led to a progressive increase in ethanol and acetaldehyde levels emitted from the juice (Table 1). The IW treatment markedly reduced off-flavor volatile emission levels, compared with those detected following continuous storage at 2 °C (Table 1). On the other hand, following the TC treatment, ethanol and acetaldehyde levels remained low after 8 and 12 weeks of storage, but significantly increased to very high levels after 16 weeks (Table 1).

EFFECTS OF IW AND TC ON FRUIT TASTE FOLLOWING LONG-TERM COLD STORAGE. Taste panels indicated that the initial taste score of 'Oroblanco' fruit at the beginning of the experiments was very high (8.5). However, in

Table 1. Effects of storage temperatures, intermittent warming (IW), and temperature conditioning (TC) on the postharvest qualities of 'Oroblanco' citrus fruit following long-term storage.

Time in storage/ treatment[z]	Fruit		Juice			
	Decay (%)	Wt loss (%)	Total soluble solids (%)	Acid (%)	Ethanol [$\mu L \cdot L^{-1}$ (ppm)]	Acetaldehyde [$\mu L \cdot L^{-1}$ (ppm)]
8 weeks						
11 °C	1.5 b	4.0 a	11.0 a	1.0 a	2 d	4 d
2 °C	16.0 a	3.6 a	11.1 a	1.0 a	108 a	76 a
IW	1.5 b	3.5 a	11.1 a	1.0 a	11 c	20 c
TC	1.6 b	4.0 a	11.2 a	1.0 a	30 b	39 b
12 weeks						
11 °C	1.5 b	5.0 a	11.3 a	1.0 a	6 c	4 d
2 °C	22.0 a	4.1 a	11.4 a	1.0 a	177 a	106 a
IW	2.0 b	4.2 a	11.1 a	1.0 a	15 c	30 c
TC	2.0 b	4.8 a	11.3 a	1.0 a	50 b	60 b
16 weeks						
11 °C	2.0 c	6.3 a	11.1 a	0.9 a	10 b	15 d
2 °C	49.0 a	4.6 b	11.0 a	0.9 a	220 a	140 b
IW	2.5 c	4.9 b	10.9 a	0.9 a	10 b	50 c
TC	23.5 b	5.2 b	11.1 a	0.9 a	260 a	220 a

[z]Fruit were stored at 11 °C (51.8 °F), 2 °C (35.6 °F), or handled under IW (storage at cycles of 3 weeks at 2 °C + 1 week at 11 °C) or TC [a prestorage treatment for 7 d at 16 °C (60.8 °F) before continuous storage at 2 °C] regimes. Measurements were taken after 8, 12, and 16 weeks. Data of decay development are means of 100 fruit per treatment, data of weight loss are means of 15 fruit per treatment and data of total soluble solids and acid percentages are means of 10 fruit per treatment. The data of ethanol and acetaldehyde levels are means of four replications taken from a total of 12 fruit per treatment. Different letters within a column indicate significant differences at $P < 0.05$, according to a Student-Newman-Keuls one-way analysis of variance test on ranks.

control fruit stored at 11 °C, it gradually declined during long-term storage to 7.9, 6.6 and 5.8 after 8, 12, and 16 weeks, respectively (Fig. 3). The progressive decrease in fruit taste was most severe in chilling-injured fruit stored continuously at 2 °C (Fig. 3). In contrast, the taste scores of IW-treated fruit remained relatively high throughout the entire long-term storage period, and were 7.6, 7.3, and 7.3 after 8, 12, and 16 weeks, respectively (Fig. 3). The taste scores of TC-treated fruit remained high (about 7.5) after 8 and 12 weeks of storage, but dramatically decreased to only 5.5 after 16 weeks of storage (Fig. 3).

Discussion

'Oroblanco' fruit are picked during a short period of time between October and November, before their peel color turns yellow, and are marketed shortly afterwards to insure that they will not become yellow during postharvest storage. In the present study, we showed that by continuous storage at a low temperature of 2 °C, the fruit retain green for a period of up to 16 weeks after harvest. Unfortunately, this treatment results in severe chilling injuries (Figs. 1 and 2). To overcome this obstacle, we further evaluated the application of IW and TC techniques to maintain fruit quality following long-term cold storage.

The data shows that by using IW (storage at cycles of 3 weeks at 2 °C + 1 week at 11 °C) it was possible to retain the green color of fruit and maintain acceptable postharvest quality for up to

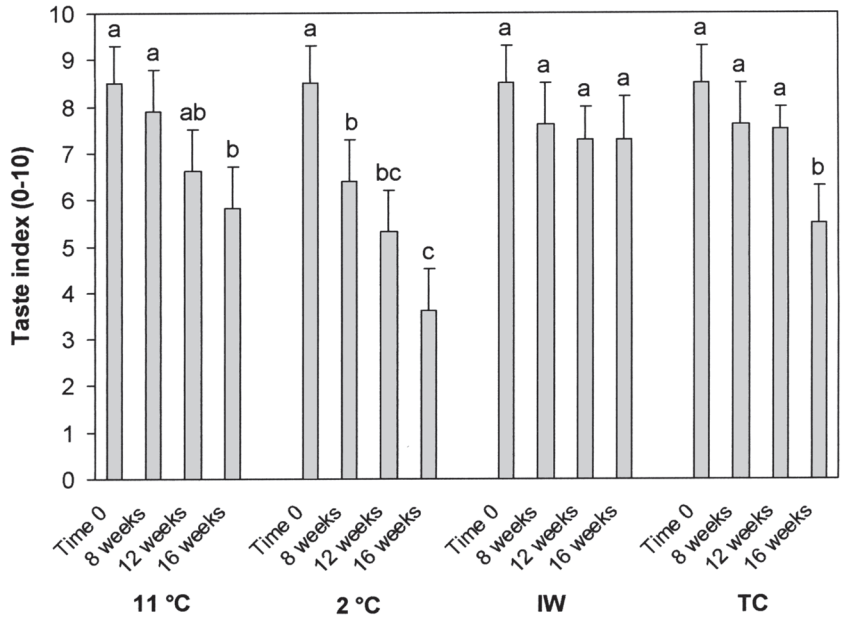

Fig. 3. Effects of storage temperatures, intermittent warming (IW), and temperature (TC) conditioning on the taste of 'Oroblanco' fruit. Fruit were stored at 11 °C (51.8 °F), 2 °C (35.6 °F), or handled under IW (storage at cycles of 3 weeks at 2 °C + 1 week at 11 °C) or TC [a prestorage treatment for 7 d at 16 °C (60.8 °F) before continuous storage at 2 °C] regimes. Taste scores were evaluated after 8, 12, and 16 weeks. Data are means ± SE of taste scores collected from 15 people. The taste score index was 0 to 10, with 0 = very objectionable and 10 = excellent. Columns marked by different letters are significantly different at $P < 0.05$ according to a Student-Newman-Keuls one way analysis of variance test on ranks.

16 weeks: chilling injury and decay levels remained low and fruit taste scores were sufficient (Figs. 1–3, Table 1).

Using TC (a prestorage treatment for 7 d at 16 °C before continuous storage at 2 °C), it was possible to keep the fruit quality high for up to 8 to 10 weeks after harvest; however, after longer periods the fruit suffered from chilling damage and decay, and off-flavor volatiles began to accumulate in the juice (Figs. 1–3, Table 1).

From a commercial point of view, it is much more easier to apply TC rather than IW, since all TC requires is to decrease the storage temperature once after 7 d of storage from 16 to 2 °C, as compared with repeated periodic temperature changes required for IW. We suggest the following options for postharvest storage of 'Oroblanco' fruit: for short periods of time (up to 4 weeks) the fruit can be stored directly in 2 °C with little risk of the development of chilling injury (Porat et al., 2001); for intermediate periods of storage (up to 8 weeks) the fruit should at least be pretreated by TC to avoid chilling damage; and for long-term storage (12 to 16 weeks) the fruit must be treated by IW. Adopting these storage technologies will make it possible to prolong the relatively short marketing period of 'Oroblanco' fruit for up to 4 months after harvest.

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