Hot Water and Elevated CO₂ Effects on Proline and Other Compositional Changes in Relation to Postharvest Chilling Injury of ‘Marsh’ Grapefruit

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ABSTRACT. Heat treatments and exposure to elevated CO₂ are known to reduce the incidence of chilling injury on grapefruit. In the current study, ‘Marsh’ grapefruit (*Citrus paradisi* Macf.) were harvested on 17 Jan. or 22 Mar. 1996 and exposed to hot water (HW) dips (48 °C for 120 minutes) or exposed to controlled atmosphere (CA) of 10% or 16% CO₂ during the first 3 weeks of an 8-week cold storage period (4.5 °C) to test their effects on the development of peel pitting (i.e., chilling injury) and proline and other compositional changes of the peel and juice. All HW and CA treatments from both harvests greatly reduced the development of peel pitting compared to the control. These treatments were also associated with lower average proline levels in the flavedo during storage. This suggests that HW and elevated CO₂ may reduce chilling-induced peel pitting by facilitating proline metabolism in grapefruit flavedo tissue. HW and CA treatments resulted in higher peel total soluble and nonreducing sugar levels, but effects on peel reducing sugar and free amino acid concentrations were not consistent. In the juice, HW reduced titratable acidity (TA) concentrations while CA tended to increase both TA and ascorbic acid concentrations. Compared to the control, CA resulted in a slight decrease in total soluble solids during storage, while the effect of HW was inconsistent.

Grapefruit, like many tropical and subtropical fruits, are chilling sensitive and are injured when held at temperatures below ≈10 °C. The sensitivity of grapefruit to chilling injury (CI) prevents storage at lower temperatures and has been recognized as a limiting factor in their long-term storage. The most common symptom of CI on grapefruit is surface pitting, which is characterized by development of circular depressions (cell collapse) on the peel surface usually developing after 2 to 3 weeks of continuous cold storage (Purvis and Grierson, 1982). The development of peel pitting in Florida grapefruit occurs most rapidly at ≈5 °C (Purvis, 1985) with much research on grapefruit CI conducted at 4.4 °C (Purvis and Grierson, 1982). Several physical and chemical treatments have been reported to reduce CI on grapefruit. However, the mechanisms of the protection afforded by these treatments are still not clear.

Postharvest diseases in tropical fruits have been effectively controlled by hot water (HW) and other forms of heat treatment (Barkai-Golan and Phillips, 1991). HW treatments also slow ripening processes, can reduce physiological disorders, and leave no chemical residues on fruit (Klein and Lurie, 1991). Many studies have shown that HW treatments also reduce the incidence of CI symptoms, such as peel pitting, on ‘Marsh’ grapefruit when held at chilling temperatures (El-Shiekh, 1996; Rodov et al., 1995; Wild, 1993; Wild and Hood, 1989). For example, Wild (1993)

reported that HW dipping (50 °C for 2 min) before storage at 1 °C significantly reduced CI on ‘Marsh’ grapefruit. El-Shiekh (1996) reported no CI during 90 d storage at 4 °C for ‘Marsh’ previously exposed to 48 °C water for 120 min or 45 °C water for 180 min compared with ≈40% CI incidence in control fruit.

Exposure to elevated levels of CO₂ during storage has long been known to improve the quality of many stored fruits and vegetables through depressed metabolic activity (Watkins, 2000). Specifically, several researchers have reported that exposure of ‘Marsh’ grapefruit to elevated CO₂ levels decreases chilling-induced peel pitting during storage at chilling temperatures (Grierson, 1974; Vakis et al., 1970; Wardowski et al., 1975). For example, Vakis et al. (1970) reported that addition of 10% CO₂ to the storage atmosphere reduced peel pitting of ‘Marsh’ grapefruit from 61% (control) to 2% after 3 weeks at 4.4 °C. Lower CO₂ concentrations gave intermediate protection from chilling-induced pitting.

Purvis and Grierson (1982), Nordby et al. (1987), and Nawar and Ezz (1994) studied CI in ‘Marsh’ grapefruit and ‘Washington’ navel orange and found a relationship between chilling sensitivity and the at-harvest concentration of proline, free amino acids, and soluble sugars in the peel. In particular, Nawar and Ezz (1994) reported that the concentration of these constituents was higher in the more chilling-resistant variety (orange) than in the more chilling-sensitive (grapefruit).

The present study was initiated to evaluate the development of peel pitting and the changes in concentration during storage of different peel constituents (proline, free amino acids, and soluble sugars) in ‘Marsh’ grapefruit in response to a prestorage HW treatment or exposure to controlled atmosphere (CA) with elevated CO₂ levels during the first 3 weeks of storage at 4.5 °C, treatments known to reduce CI. In addition, compositional changes in the juice [ascorbic acid, titratable acidity (TA), and total soluble solids (TSS)] were measured to evaluate effects of the pretreatments and CI on internal quality.

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Materials and Methods

'Marsh' grapefruit of uniform size (≈ 106 to 121 mm diameter) growing on sour orange rootstock in Fellsmere, Fla., were harvested by hand on either 17 Jan. or 22 Mar. 1996 from the exterior canopy of healthy trees. After harvest, fruit were immediately transported by car to the postharvest laboratory at the Univ. of Florida in Gainesville. Each harvest represented a separate experiment. Fruit were washed by hand with a sponge and deionized water (no detergent), then air-dried and randomly distributed among the treatments. Each treatment $\times$ storage time combination consisted of nine, single-fruit replicates in nylon mesh drawstring bags.

Fruit were exposed to the following treatments: 1) submersion in 48°C water for 120 min before storage, 2) exposure to 10% CO_2 during the first 3 weeks of storage, 3) exposure to 16% CO_2 during the first 3 weeks of storage, or 4) control fruit that received no heat or elevated CO_2 treatments. Fruit from all treatments were stored in flowing systems (see CA Treatments below) at 4.5°C and $>95\%$ relative humidity (RH) for the first 3 weeks of storage; and subsequently in the open storage room with $90\% \pm 5\%$ RH for the remainder of the 8-week storage period.

HOT WATER TREATMENTS. Fruit in mesh bags were treated with 48°C water for 120 min using a laboratory scale fruit heating system (model HWH-2; Gaffney Engineering, Gainesville, Fla.) capable of maintaining water temperatures up to 55°C , to a stability within $<2.0^\circ\text{C}$ of the initial water temperature for the first 4–5 min after submerging up to 32 kg of fruit with an initial fruit temperature of 20°C . Thereafter, the system is capable of maintaining the water to a stability within $\pm 0.1^\circ\text{C}$. After the HW treatment, fruit were air-dried using an electric fan at 4.5°C for 120 min before being placed in chambers (see below).

CA TREATMENTS. Grapefruit stored in elevated CO_2 at 4.5°C were placed in gas-tight chambers (Chace et al., 1969) and a constant flow of humidified gas mixtures consisting of air plus CO_2 supplied so that the CO_2 concentration was maintained at either 10% or 16% depending on the treatment. Control and HW-treated fruit were stored in similar chambers supplied with humidified air at a flow rate sufficient to maintain respiratory CO_2 concentrations below 0.5%. Fruit were held under these conditions for 3 weeks and then all were transferred to room air at 4.5°C for the remainder of the experiment. The fruit (in mesh bags) were randomly distributed among 56-L plastic slotted harvest lugs (model C1471; LINPAC, Georgetown, Ky.) on metal storage racks. Carbon dioxide concentrations were measured using a Gow-Mac model 580 GC (Gow-Mac Instr. Co., Bridgewater, N.J.) equipped with a 1020×3.12 -mm 80/100 mesh Porapak Q column at 40°C ; the thermal conductivity detector and injector were set at 90°C .

PITTING AND COMPOSITIONAL ANALYSIS. Fruit quality and composition were evaluated every 1 or 2 weeks during storage. Individual fruit (reps) were evaluated for pitting, fruit weight, and the concentrations of ascorbic acid, TSS, and TA in the juice. The peel was removed and free amino acids, and total and reducing sugar contents were determined using three composite peel samples (flavedo and albedo) from three fruit each. Proline content was evaluated using only the flavedo. The pitted area (CI symptoms) on the peel of each fruit was determined by tracing the outlines of the pits on paper and then measuring the area (Nawar and Ezz, 1994).

Soluble sugars were extracted from 15 g of the composite peel samples using 85 mL of 95% ethanol. Reducing sugars

were determined by the Somogyi (1952) method using copper reagent and measuring absorbance at 565 nm. Total sugars were determined by the colorimetric phenol method (Dubois et al., 1956) measuring absorbance at 490 nm and expressed as glucose equivalents. Nonreducing sugar content was calculated by taking the difference between total and reducing sugars. Total free amino acids were measured in the ethanol extract using the methodology described by Lee and Takahashi (1966) and modified by Nawar and Ezz (1994). Proline was extracted and determined by homogenizing 1 g of the flavedo only (no albedo) in 20 mL of 3% sulfosalicylic acid as described by Bates et al. (1973) and modified by Nawar and Ezz (1994).

Ascorbic acid, TSS, and TA contents were measured in the juice extracted from the fruit. Total ascorbic acid (mg/100 mL of juice) was determined using the dinitrophenylhydrazine method (Terada et al., 1978) measuring the absorbance at 540 nm. The TSS was measured using a temperature-compensated Abbe refractometer (model Mark II; Cambridge Instrument Co., Buffalo, N.Y.). TA (% anhydrous citric acid) was measured by titrating juice samples to pH 8.1 with NaOH using an automatic titrimer (Fisher Scientific Co., Pittsburgh).

Analysis of variance was used to determine the significance of treatments and storage time and their interactions and LSD values were calculated. Significant differences between harvests/experiments were determined by *t* tests. Data were analyzed using the Statistical Analysis System computer package (SAS Institute, 1982).

Results and Discussion

HARVEST AND STORAGE-RELATED CHANGES IN PEEL PITTING. Untreated 'Marsh' grapefruit from the first (17 Jan.) harvest developed more than twice the area of pitting (CI symptom) than fruit from the second (22 Mar.) harvest (Table 1). Grierson and Hatton (1977) reported that early- and late-season grapefruit are particularly susceptible to CI, but that Florida grapefruit become resistant to CI sometime between December and March. They found that the specific period of grapefruit resistance to CI varied greatly in magnitude, timing, and duration from year to year such that fruit susceptibility to CI could even be greater in January compared to March. Grierson (1974) and Grierson and Hatton (1977) suggested that transient midseason resistance might be related to the growth activity of the tree and to endogenous levels of unidentified plant growth regulators at the time of harvest.

As expected, the area of pitting on control fruit increased as storage time at 4.5°C progressed (Tables 2 and 3). Pitting increased rapidly after 3 or 6 weeks of storage for fruit from the first or second harvest, respectively. HW and elevated CO_2 treatments both drastically reduced the amount of peel pitting that developed by more than 10-fold, and often delayed the onset of pitting by 2 weeks or more. There were no consistent differences in peel pitting between the HW, 10%, or 16% CO_2 treatments from either harvest.

EFFECTS OF HEAT TREATMENT ON PITTING. Prestorage HW treatments reduced peel pitting area at the end of storage to a maximum of only 5.4% and 2.8% of the control fruit in the first and second harvests, respectively (Tables 2 and 3). This agrees with numerous reports showing reduced CI of citrus after various heat treatments (El-Shiekh, 1996; Rodov et al., 1995; Schirra et al., 1997; Schirra and D'hallewin, 1997; Wild and Hood, 1989). The HW treatment used in this study did not result in visible injury to the peel of fruit from either harvest. Specific heat treatments (time

Table 1. Effect of harvest date on the development of peel pitting (CI injury) and content of proline, free amino acids, and soluble sugars. Area of fruit peel pitting was measured on untreated fruit after storage for 7 or 8 weeks at 4.5 °C for fruit harvested on 17 Jan. and 22 Mar., respectively. Proline, free amino acids, reducing sugars, nonreducing sugars, and total sugars were measured at harvest.

Harvest date	Pitting area (cm ² /fruit)	Peel					Juice		
		Proline	Free amino acids	Soluble sugars			Ascorbic acid	TA	TSS
				Total	Reducing	Non-reducing			
(mg·g ⁻¹ peel fresh wt)									
17 Jan.	8.20	1.44	19.29	37.12	5.27	31.85	42.19	1.29	8.00
22 Mar.	2.86	1.78	20.71	36.56	6.98	29.58	43.79	1.29	8.20
<i>t</i> test	***	*	NS	NS	***	NS	*	NS	*

NS, *, ***Nonsignificant or significant A *P* < 0.05 or 0.001, respectively.

× temperature) need to be developed for each growing region and for different times of the year because other reports have noted that citrus sensitivity to HW treatments can change dramatically throughout the season (Schirra et al., 1997).

EFFECTS OF ELEVATED CO₂ ON PITTING. Exposure to either 10% or 16% CO₂ during the first 3 weeks of cold storage controlled peel pitting to <0.25 cm² per fruit regardless of harvest date or the amount of chilling-induced pitting on control fruit. The maximum area of peel pitting at the end of storage on fruit exposed to elevated CO₂ was only 2.4% and 1.7% of control fruit in the first and second harvests, respectively. There are many reports of elevated CO₂ levels in modified atmosphere or CA reducing CI-related peel pitting of grapefruit (Hatton et al., 1972; Vakis et al., 1970; Wardowski et al., 1975). Wardowski et al. (1975) reported that, while exposure to high CO₂ levels (up to 20%) for 32 d at 4.5 °C reduced CI in early-season (November) and mid-season (January) grapefruit, it enhanced CI of late-season (March) fruit. In the present study, CI on fruit harvested in March was not enhanced by either of the CO₂ treatments. With the variability in seasonal timing of CI susceptibility and related physiological/developmental changes already mentioned, fruit from the second harvest apparently had not yet developed the late-season onset of enhanced CI susceptibility and CO₂ sensitivity (Table 3).

PROLINE. Initial flavedo proline levels were 23% higher at the second harvest (22 Mar.) compared to the first (17 Jan.) (Table 1). This agrees with Purvis (1981) who found that proline content in grapefruit peel rose in February, when field temperatures were low, peaked in March, and then declined slowly through May, at which time they still had not reached initial levels. Such increases in proline content occurred well before the fruit developed resistance to CI, suggesting that proline alone was not directly responsible for chilling resistance.

During storage, average flavedo proline content was highest in untreated 'Marsh' grapefruit from both harvests compared to HW- or CO₂-treated fruit (Tables 2 and 3). Changes in flavedo proline content during cold storage were significant, but not consistent. Free proline content represents a balance between proline synthesis and utilization (Boggess and Stewart, 1980) and future experiments should investigate how storage at chilling temperatures and postharvest treatments specifically affect proline synthesis, degradation, and utilization. Thus, the higher flavedo proline content in control fruit could have been due to either enhanced protein degradation at chilling temperatures, and/or proline synthesis, while lower proline content in the flavedo of HW- or CO₂-treated fruit could represent proline degradation or incorporation into newly synthesized proteins.

Based on fruit samples from the field, Nawar and Ezz (1994),

Nordby et al. (1987), and Purvis (1981) found that peel proline levels were higher in fruit subsequently more resistant to CI during storage. In those cases, peel proline content was likely influenced by preharvest environmental conditions, most notably low temperatures (Purvis, 1981). Previous reports suggest that proline is primarily produced in the leaves and then transported to the fruit (Purvis and Yelenosky, 1982). Higher levels of proline at harvest may provide resources needed to better protect cellular constituents during storage at chilling temperatures. After harvest, Purvis and Yelenosky (1982) reported that flavedo proline content did not change during 4 weeks at 5 °C, though data were not presented. Nawar and Ezz (1994) reported that peel proline levels of detached navel orange and 'Marsh' grapefruit tended to increase during storage at 4.5 °C for 10 weeks, but the increases were not significant. Neither of the above studies included fruit stored at nonchilling temperatures to determine how postharvest chilling temperatures affect proline content compared to nonchilling temperatures. The present study is the first we are aware of to investigate the effects of postharvest treatments that reduce CI on flavedo proline content during storage. Here we found that postharvest treatments that reduce CI (HW and CO₂) were associated with lower average flavedo proline levels during storage at chilling temperatures (Tables 2 and 3). It is possible that one of the ways that postharvest HW and CO₂ treatments reduce CI is by enhancing proline utilization during storage at chilling temperatures to create proteins with enhanced stability at low temperatures (Steponkus, 1971).

AMINO ACIDS. The concentration of free amino acids in the peel of freshly harvested fruit did not change significantly between the first and second harvests (Table 1). Average free amino acid content during storage was only significantly different from the control in the peel of 16% CO₂-treated fruit from the first harvest (Tables 2 and 3). In this case, average free amino acid levels were 42% above the control. Thus, within the free amino acid concentrations encountered in these studies, free amino acid content in the peel does not appear to play a direct role in determining the tissue's susceptibility to CI-induced peel pitting. The content of free amino acids in the peel increased markedly after 1 or 2 weeks of storage at 4.5 °C and fluctuated thereafter in fruit from both harvests. When exposed to chilling temperatures of 0 to 10 °C, free amino acid content in tissues of subtropical plants has been reported to increase along with a 4- to 9-fold increase in protein hydrolysis (Leopold and Kriedemann, 1981; Levitt, 1969). Steponkus (1971) reported that during cold acclimation, plants produce proteins with a higher sugar-binding capacity, which protects the protein from being denatured at low temperatures. Thus, the breakdown in certain proteins may free up amino acids

Table 2. Effects of hot water (48 °C for 2 h) or elevated CO₂ (10% or 16%) on fruit peel pitting area, compositional changes, and weight loss of 'Marsh' grapefruit harvested 17 Jan. 1996 and stored at 4.5 °C for 7 weeks. Fruit were exposed to HW before storage or elevated CO₂ during the first 3 weeks of storage. Free A.A. = free amino acids; TA = titratable acidity (% anhydrous citric acid); and TSS = total soluble solids.

Treatment	Weeks @ 4.5 °C	Pitting area (cm ² /fruit)	Peel					Juice		
			Proline	Free a.a.	Soluble sugars			Ascorbic acid (mg/100 mL)	TA (%)	TSS (%)
					Total	Reducing	Non-red.			
Control	0	0.00	1.44	19.29	37.12	5.27	31.85	42.19	1.29	8.00
	1	0.00	3.65	44.44	55.36	6.69	48.67	41.44	1.29	8.04
	2	0.00	2.76	36.09	62.64	11.69	50.95	38.96	1.27	8.03
	3	2.47	2.25	33.78	76.48	15.85	60.63	30.80	1.25	8.10
	4	3.45	1.44	28.44	55.36	6.77	48.59	32.35	1.19	8.10
	6	7.82	2.35	31.20	68.96	19.40	49.56	31.62	1.08	8.20
	7	8.20	2.86	60.18	53.60	21.47	32.16	27.60	1.06	8.50
	Mean	3.13	2.39	36.20	58.50	12.44	46.06	34.99	1.21	8.14
Hot Water	0	0.00	1.44	19.29	37.12	5.27	31.85	42.19	1.29	8.00
	1	0.00	1.38	30.67	51.20	5.49	45.71	41.18	1.25	8.04
	2	0.00	1.43	28.18	60.72	6.10	54.62	37.50	1.20	8.11
	3	0.00	2.47	37.33	62.40	7.20	55.29	32.10	1.15	8.00
	4	0.00	2.19	50.76	82.80	13.60	69.20	30.23	1.14	8.10
	6	0.37	1.31	25.87	70.08	17.72	52.36	28.37	1.09	8.17
	7	0.44	1.59	34.84	80.16	12.67	67.48	24.90	1.10	8.30
	Mean	0.12	1.69	32.42	63.50	9.71	53.79	33.78	1.17	8.10
10% CO ₂	0	0.00	1.44	19.29	37.12	5.27	31.85	42.19	1.29	8.00
	1	0.00	1.04	65.69	71.84	13.02	58.82	39.50	1.29	8.00
	2	0.00	1.02	34.04	77.20	5.40	71.80	41.15	1.27	7.90
	3	0.00	1.25	30.58	81.44	17.77	63.67	37.70	1.25	8.10
	4	0.00	2.11	45.33	80.40	7.88	72.52	32.56	1.20	8.04
	6	0.18	1.46	39.73	80.24	16.37	63.87	28.59	1.19	8.16
	7	0.20	1.45	46.84	81.68	15.52	66.16	28.30	1.09	8.20
	Mean	0.06	1.40	40.22	72.85	11.60	61.24	35.71	1.23	8.06
16% CO ₂	0	0.00	1.44	19.29	37.12	5.27	31.85	42.19	1.29	8.00
	1	0.00	1.85	54.22	76.56	11.54	65.02	41.66	1.35	8.00
	2	0.00	2.14	64.62	83.04	11.00	72.04	42.76	1.29	7.94
	3	0.00	2.02	44.62	71.12	8.93	62.16	36.70	1.28	7.96
	4	0.00	2.15	73.78	71.52	6.41	65.11	34.28	1.24	8.09
	6	0.00	1.80	62.40	71.44	14.73	56.71	31.09	1.22	8.23
	7	0.04	2.03	40.18	78.32	16.93	61.31	28.90	1.20	8.10
	Mean	0.01	1.92	51.30	69.87	10.69	59.17	36.80	1.27	8.05
Overall L.S.D. (0.05)		0.12	0.16	4.00	2.81	0.80	2.72	0.94	0.01	0.05
ANOVA										
Treatment		***	***	***	***	***	***	***	***	***
Week		***	***	***	***	***	***	***	***	***
Treatment x Week		***	***	***	***	***	***	***	***	**

, *Significant at *P* < 0.01 or 0.001, respectively.

that are used to create new proteins that are more stable at cold temperatures.

SOLUBLE SUGARS. Initial concentration of total sugars in the peel of grapefruit was not different between the first and second harvest dates (Table 1). Purvis and Grierson (1982) reported that total soluble carbohydrates content in the peel of grapefruit more than doubled between October and February and then dropped so that levels in March were only slightly higher than those in January. During storage at 4.5 °C, total soluble carbohydrate

levels in the peel increased substantially within the first week, after which time levels generally remained high (Tables 2 and 3). Others have reported no significant increase in total carbohydrate peel content during storage at low temperatures (Nawar and Ezz, 1994; Purvis, 1989; Purvis and Yelenosky, 1982).

HW and CA treatments from both harvest dates consistently resulted in greater total peel carbohydrate levels than the control. HW (Schirra and D'hallewin, 1997) and high CO₂ (Kader, 2002) treatments have been shown to reduce respiration, which

Table 3. Effects of hot water (48 °C for 2 h) or elevated CO₂ (10% or 16%) on fruit peel pitting area, compositional changes, and weight loss of 'Marsh' grapefruit harvested 22 Mar. 1996 and stored at 4.5 °C for 8 weeks. Fruit were exposed to HW before storage or elevated CO₂ during the first 3 weeks of storage. Free A.A. = free amino acids; TA = titratable acidity (% anhydrous citric acid); and TSS = total soluble solids.

Treatment	Weeks @ 4.5 °C	Pitting area (cm ² /fruit)	Peel					Juice			Weight loss (%)
			Proline	Free a.a.	Soluble sugars			Ascorbic acid (mg/100 mL)	TA (%)	TSS (%)	
					Total	Reducing	Non-red.				
Control	0	0.00	1.78	20.71	36.56	6.98	29.58	43.79	1.29	8.20	0.00
	1	0.00	1.83	32.80	70.00	8.93	61.07	41.32	1.29	8.28	0.80
	2	0.00	1.94	38.13	63.20	11.70	51.50	40.16	1.29	8.32	1.87
	4	0.00	2.14	30.84	37.44	11.55	25.89	33.02	1.21	8.34	1.70
	6	1.63	2.60	45.69	73.36	13.94	59.42	29.67	1.08	8.41	2.72
	8	2.86	1.76	34.93	72.00	15.47	56.52	27.50	1.03	8.60	3.10
	Mean	0.75	2.01	33.85	58.76	11.43	47.33	35.91	1.20	8.36	1.70
Hot Water	0	0.00	1.78	20.71	36.56	6.98	29.58	43.79	1.29	8.20	0.00
	1	0.00	2.20	44.71	56.88	13.28	43.60	43.50	1.22	8.58	1.10
	2	0.00	2.18	55.82	70.32	16.20	54.12	40.58	1.19	8.78	2.21
	4	0.00	1.51	30.93	77.12	13.56	63.56	32.07	1.18	8.69	2.72
	6	0.08	1.06	32.71	70.88	14.55	56.33	29.82	1.02	8.66	3.07
	8	0.08	1.50	37.42	81.20	18.93	62.27	26.20	1.00	8.80	4.69
	Mean	0.03	1.70	37.05	65.49	13.92	51.58	35.99	1.15	8.62	2.30
10% CO ₂	0	0.00	1.78	20.71	36.56	6.98	29.58	43.79	1.29	8.20	0.00
	1	0.00	1.33	34.22	79.44	6.87	72.57	41.90	1.25	8.11	0.71
	2	0.00	1.41	40.27	78.80	8.50	70.30	40.04	1.23	8.11	1.66
	4	0.00	1.57	25.51	64.72	11.30	53.42	34.26	1.20	8.22	1.74
	6	0.00	2.33	43.29	71.36	11.89	59.47	33.28	1.16	8.23	2.27
	8	0.25	2.13	44.18	75.68	16.67	59.00	29.10	1.00	8.30	3.28
	Mean	0.05	1.76	34.70	67.76	10.37	57.39	37.07	1.19	8.20	1.61
16% CO ₂	0	0.00	1.78	20.71	36.56	6.98	29.58	43.79	1.29	8.20	0.00
	1	0.00	1.54	34.58	74.64	7.37	67.27	42.34	1.28	8.04	0.60
	2	0.00	0.93	25.60	70.40	11.11	59.29	41.38	1.28	8.00	1.55
	4	0.00	1.50	26.93	57.04	15.82	41.22	39.32	1.22	7.96	1.49
	6	0.06	1.91	54.93	70.64	15.41	55.23	35.26	1.21	8.06	2.18
	8	0.12	2.08	50.13	74.08	13.33	60.65	30.90	1.10	8.00	3.18
	Mean	0.03	1.62	35.84	63.89	11.67	52.21	38.83	1.23	8.04	1.50
Overall L.S.D. (0.05)		0.11	0.13	3.63	2.74	0.40	2.61	0.58	0.01	0.07	0.99
ANOVA											
Treatment		***	***	ns	***	***	***	***	***	***	***
Week		***	***	***	***	***	***	***	***	***	***
Treatment x Week		***	***	***	***	***	***	***	***	***	***

ns, ***Nonsignificant or significant at $P < 0.001$, respectively.

reduces carbohydrate demand and may help explain why these treatments resulted in greater total carbohydrate content in the peel compared to control fruit.

Initial concentration of reducing sugars in the peel of grapefruit was slightly higher in the first harvest date while nonreducing sugars (primarily sucrose) did not change significantly (Table 1). For both harvest dates, the concentration of reducing sugars fluctuated from week to week but generally increased throughout storage at 4.5 °C (Tables 2 and 3). HW- and CO₂-treated grapefruit peel from the first harvest contained lower average amounts of reducing sugars compared with control fruit. In the second harvest, reducing sugars were again lower in the peel of fruit exposed to 10% CO₂, but fruit exposed to 16% CO₂ were not significantly different from the control. HW treatment resulted in an increase in reducing sugars. For both harvests, nonreducing sugars increased 1.5- to 2-fold during the first week of storage at 4.5 °C and then generally leveled off and fluctuated through the remainder of storage. Both HW and CO₂ treatments resulted in

higher average levels of nonreducing sugars in the peel.

Reducing sugars in the peel of grapefruit attached to the tree have been reported to increase and sucrose content decrease when weekly mean minimum field temperatures drop below 10 °C (Purvis and Grierson, 1982) or during low temperature (e.g., 5 °C) storage of detached fruit (Nawar and Ezz, 1994; Purvis, 1989; Purvis and Yelenosky, 1982). Increases in the content of reducing sugars and decreases in sucrose content were related to increased invertase activity in grapefruit flavedo (Purvis and Rice, 1983). The changes in reducing sugars reported here generally agree with those reported above. Changes in sucrose content reported by others are not comparable to changes in nonreducing sugars reported here because the proportion of sucrose included with nonreducing sugars may vary widely (e.g., from 25% to 75%; Purvis, 1989). Furthermore, exposed grapefruit fruit from the exterior canopy are more chilling sensitive than fruit from the interior canopy, but peel concentrations of sucrose and reducing sugars were similar at harvest and during storage, suggesting that reducing sugars

may not play a direct role in determining the tissue's sensitivity to chilling temperatures (Purvis, 1980, 1989).

ASCORBIC ACID. Ascorbic acid content in the juice was slightly higher in the first harvest (Table 1). Storage at 4.5 °C always resulted in a loss of ascorbic acid over time (Tables 2 and 3). Control fruit lost 17% or 18% of their ascorbic acid content after 7 or 8 weeks of storage, respectively. Only the 16% CO₂ treatment from both harvests significantly slowed the loss of ascorbic acid, with fruit losing between 11% and 13% of their ascorbic acid content during storage. The 10% CO₂ and HW treatments did not result in consistent differences from the control. The results presented here agree with other reports that tissue ascorbic acid levels decline during storage and when exposed to chilling temperatures or in response to commodity water loss (Lee and Kader, 2000).

TITRATABLE ACIDS. There was no difference in initial fruit TA between the first and second harvests (Table 1). The TA declined during storage for all treatments, representing an 18% to 20% drop in control fruit during 7 or 8 weeks storage at 4.5 °C (Tables 2 and 3). For both harvests, exposure to 16% CO₂ significantly delayed and reduced the magnitude of the drop in TA levels during storage. Exposure to 10% CO₂ also significantly delayed the decline in TA in fruit from the first harvest, but did not for the second harvest. HW treatment tended to result in a more rapid decline in TA initially during storage, but by the end of the storage period TA was not consistently different from the control.

TOTAL SOLUBLE SOLIDS AND WEIGHT LOSS. Total soluble solids in the juice increased slightly between the first and second harvests as the fruit continued to mature on the tree (Table 1). After 7 or 8 weeks of storage at 4.5 °C, fruit TSS usually increased slightly (Tables 2 and 3) as the fruit lost weight (Table 3) and sugars were concentrated. Some of the measured increase in TSS may have also been due to solubilization of compounds other than carbohydrates. Echeverria and Ismail (1990) reported that while °Brix (equivalent to TSS) increased in 'Marsh' grapefruit from 10.2 to 10.5 after 7 weeks at 15 °C, total sugars actually decreased 7.2%. Hydrolysis of cell-wall constituents could also possibly contribute to the observed increase in °Brix (Burns, 1990). Average TSS levels during storage were lower in CO₂-treated fruit than in control or HW-treated fruit even though weight loss was equal to the control (Tables 2 and 3). The TSS of HW-treated fruit was only significantly different from the control after the second harvest when they also had the greatest weight loss after 8 weeks of storage, representing a 51% increase over the control (Table 3). Others have also reported enhanced water loss from HW-treated fruit (Schirra and D'hallewin, 1997).

The mechanism of HW- or CO₂-induced CI resistance remains unresolved. In our work reported here, these treatments resulted in reduced flavedo proline and increased peel total soluble and nonreducing sugar content. These changes may be related to the breakdown in chilling-labile proteins and the formation of chilling-stable proteins that may bind sugar molecules to enhance stability. The low levels of proline and reducing sugars that were associated with chilling resistant tissue in storage in this study contrast with the data from Purvis (1981, 1989) indicating that seasonal increases in CI resistance correspond to elevated levels of those constituents and thus suggest that levels of proline and soluble sugars alone are not responsible for reduced CI in HW- or CO₂-treated fruit.

Literature Cited

Barkai-Golan, R. and D. Phillips. 1991. Postharvest heat treatment for fresh fruits and vegetables for decay control. *Plant Dis.* 75:1085–1089.

- Bates, L.S., R.P. Walder, and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207.
- Bogges, S.F. and C.R. Stewart. 1980. The relationship between water stress induced proline accumulation and inhibition of protein synthesis in tobacco leaves. *Plant Sci. Lett.* 17:245–252.
- Burns, J.K. 1990. α - and β -galactosidase activities in juice vesicles of stored Valencia oranges. *Phytochemistry* 29:2425–2429.
- Chace, Jr., W.G., P.L. Davis, and R.H. Cubbedge. 1969. An automatic gas chromatograph and gas-handling system for controlled-atmosphere storage. *Proc. Natl. Controlled Atmosphere Res. Conf., Mich. State Univ.*, p. 1–9.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350–356.
- Echeverria, E. and M. Ismail. 1990. Sugars unrelated to Brix changes in stored citrus fruit. *HortScience* 25:710.
- El-Shiekh, A.F. 1996. Effect of different postharvest hot water treatments on quality and storability of 'Marsh' grapefruit. *Gartenbauwissenschaft* 61:91–95.
- Grierson, W. 1974. Chilling injury in tropical and subtropical fruit. V. Effect of harvest date, degreening, delayed storage and peel color on chilling injury of grapefruit. *Proc. Trop. Region. Amer. Soc. Hort. Sci.* 18:66–73.
- Grierson, W. and T.T. Hatton. 1977. Factors involved in storage of citrus fruits: A new evaluation. *Proc. Intl. Soc. Citricult.* 1:227–231.
- Hatton, Jr., T.T., J.J. Smoot, and R.H. Cubbedge. 1972. Influence of carbon dioxide exposure on stored mid- and late-season 'Marsh' grapefruit. *Proc. Trop. Reg. Amer. Soc. Hort. Sci.* 16:49–58.
- Kader, A.A. 2002. Modified atmospheres during transport and storage, p. 135–144. In: A.A. Kader (ed.). *Postharvest technology of horticultural crops*. Univ. Calif. Div. Agr. Nat. Res., Publ. 3311, Berkeley, Calif.
- Klein, J.D. and S. Lurie. 1991. Postharvest heat treatment and fruit quality. *Postharvest News Info.* 2:15–19.
- Lee, S.K. and A.A. Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20:207–220.
- Lee, Y.P. and T. Takahashi. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* 14:71–77.
- Leopold, A.C. and P.E. Kriedemann. 1981. *Plant growth and development*. Tate McGraw-Hill, New Delhi, India.
- Levitt, J. 1969. Growth and survival of plants at extremes of temperature: A unified concept. *Symp. Soc. Expt. Biol.* 23:395–448.
- Nawar, A. and T. Ezz. 1994. Surface pitting, proline, free amino acids and soluble sugars in grapefruit and orange fruit peel in relation to chilling injury. *Alex. J. Agr. Res.* 39(1):357–371.
- Nordby, H.E., A.C. Purvis, and G. Yelenosky. 1987. Lipids in peel of grapefruit and resistance to chilling injury during cold storage. *HortScience* 22:915–917.
- Purvis, A.C. 1980. Influence of canopy depth on susceptibility of 'Marsh' grapefruit to chilling injury. *HortScience* 15:731–733.
- Purvis, A.C. 1981. Free proline in peel of grapefruit and resistance to chilling injury during cold storage. *HortScience* 16:160–161.
- Purvis, A.C. 1985. Relationship between chilling injury of grapefruit and moisture loss during storage: Amelioration by polyethylene shrink film. *J. Amer. Soc. Hort. Sci.* 110:385–388.
- Purvis, A.C. 1989. Soluble sugars and respiration of flavedo tissue of grapefruit stored at low temperature. *HortScience* 24:320–322.
- Purvis, A.C. and W.G. Grierson. 1982. Accumulation of reducing sugar and resistance of grapefruit peel to chilling injury as related to winter temperatures. *J. Amer. Soc. Hort. Sci.* 107:139–142.
- Purvis, A.C. and J.D. Rice. 1983. Low temperature induction of invertase activity in grapefruit flavedo tissue. *Phytochemistry* 22:831–834.
- Purvis, A.C. and G. Yelenosky. 1982. Sugar and proline accumulation in grapefruit flavedo and leaves during cold hardening of young trees. *J. Amer. Soc. Hort. Sci.* 107:222–226.
- Rodov, V., S. Ben-Yehoshua, R. Albagli, and D.Q. Fang. 1995. Reducing chilling injury and decay of stored citrus fruit by hot water dips. *Postharvest Biol. Technol.* 5:119–127.

- SAS Institute. 1982. SAS user's guide: Statistics. SAS, Cary, N.C.
- Schirra, M., M. Agabbio, G. D'hallewin, M. Pala, and R. Ruggiu. 1997. Response of Tarocco oranges to picking date, postharvest hot water dips, and chilling storage temperature. *J. Agr. Food Chem.* 45:3216–3220.
- Schirra, M. and G. D'hallewin. 1997. Storage performance of Fortune mandarins following hot water dips. *Postharvest Biol. Technol.* 10:229–238.
- Somogyi, M. 1952. Notes on sugar determination of sugars and related substances. *Anal. Chem.* 28:350–356.
- Steponkus, P.L. 1971. Cold acclimation of *Hedera helix*. Evidence for a two-phase process. *Plant Physiol.* 47:175–180.
- Terada, M., Y. Watanabe, M. Kunitoma, and E. Hayashi. 1978. Differential rapid analysis of ascorbic acid and ascorbic acid 2-sulfate by dinitrophenylhydrazine method. *Anal. Biochem.* 84:604–608.
- Vakis, N., W. Grierson, and J. Soule. 1970. Chilling injury in tropical and subtropical fruits. III. The role of CO₂ in suppressing chilling injury of grapefruit and avocados. *Proc. Trop. Reg. Amer. Soc. Hort. Sci.* 14:89–100.
- Wardowski, W.F., L.G. Albrigo, and W. Grierson. 1975. Chilling injury and decay of grapefruit as affected by thiabendazole, benomyl, and CO₂. *HortScience* 10:381–383.
- Watkins, C.B. 2000. Responses of horticultural commodities to high carbon dioxide as related to modified atmosphere packaging. *Hort-Technology* 10:501–506.
- Wild, B.L. 1993. Reduction of chilling injury in grapefruit and oranges stored at 1°C by pre-storage hot dip treatment, curing, and wax application. *Austral. J. Expt. Agr.* 33:495–498.
- Wild, B.L. and C.W. Hood. 1989. Hot dip treatments reduce chilling injury in long-term storage of 'Valencia' oranges. *HortScience* 24:109–110.