Treating 'Primofiori' Lemons in Cold Storage with Intermittent Warming and Carbon Dioxide

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Abstract. Several intermittent 13C warming treatments were applied to 'Primofiori' lemons (*Citrus limon* Burn) stored at 2 or 8C. Fruit stored at 13C were treated with 10%, 20%, or 30% CO₂ for 24 hours at weekly intervals. Reduction in decay and physiological disorders was best with two cycles of 2 weeks at 2C and 2 weeks at 13C and relative humidity >95%. Under this storage condition, soluble solids concentration, pH, titratable acidity, and reducing sugars did not change relative to values at harvest, but the concentration of ascorbic acid increased and that of nonreducing sugars decreased in relation to harvest values. Carbon dioxide treatments did not prevent the development of alternaria (*Alternaria citri* Ell. & Pierce) rot and red blotch disorder, but effectively prevented the development of membranosis, rind pitting, and oleocellosis.

Several techniques have been applied to alleviate chilling injury (CI) in lemons stored for 2 to 6 months in relative humidities (RHs) >90%. These techniques include using moderate temperatures ($\approx$ 13C), controlled-atmosphere storage, intermittent fruit warming during cold storage, applying 2-(4-thiazolyl-benzimidazole) (thiabendazole) and methyl { 1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl } carbamate (benomyl), prestorage curing at higher temperatures, CO₂ treatments, and us-

ing semipermeable plastic films.

Storing 'Verna', 'Primofiori', 'Fino', 'Eureka', and 'Villa-Franka' lemons at 12 to 14C usually induces physiological disorders (Artés and Carpena, 1977; Artés et al., 1979, 1981; Chalutz et al., 1981; Cohen et al., 1983; Guillén et al., 1980). The main disorders are red blotch or adustiosis, peteca, membranosis or membranous stain, oleocellosis, and rind pitting or deep brown spot (Artés and Escriche, 1988; Artés et al., 1981). Disorder severity is related to cultivar, environmental conditions at picking, fruit maturity, and postharvest treatments (temperature, RH, ventilation, packinghouse treatments, and storage duration).

Inconsistent results have been reported on the effectiveness of controlled-atmosphere storage in reducing CI (Wang, 1982). 'Primofiori' lemons exposed to 12% O₂ and

<2% CO₂ at 13C and RH >95% gave the best control of physiological disorders during 2 months of storage (Artés et al., 1981; Guillén et al., 1980).

Davis and Hoffmann (1973) suggested intermittent warming to reduce citrus fruit CI. Intermittently warming 'Villa-Franka' lemons for 7 days at 13C every 21 days during storage at 2 or 8C eliminated membranosis, prevented rind pitting, and reduced decay compared with storing lemons continuously at 2 or 8C for 6 months (Cohen et al., 1983). Good results also were obtained in commercial practice (Cohen, 1988; Cohen et al., 1990). Intermittent warming for 7 days at 13C after 21 days at 2C prevented disorders in 'Fine' lemons (Artés et al., 1990).

Paraffin at 25 or 50 g-liter⁻¹ and benomyl at 0.2 g-liter⁻¹ or thiabendazole at 4 g-liter⁻¹ or 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4(1H,3H)-pyrimidinedione (iprodione) at 0.2 g-liter⁻¹ reduced red blotch disorder but not membranosis in 'Primofiori' lemons stored at 13C and 90% RH for 2 months (Artés et al., 1983).

Conditioning lemons for 1 week at 15C before storing them at 1C significantly reduced CI incidence and severity (Houck et al., 1990). Prestorage treatments with high CO₂ concentrations (10%, 20%, or 40%) for 3 or 7 days at 21C significantly reduced rind pitting in grapefruit stored at 4.4C for 8 or 12 weeks (Hatton et al., 1975). Seal-packaging individual fruit in high-density polyethylene plastic film prevented or reduced CI in lemon (Ben-Yehoshua et al., 1977; Cohen et al., 1990).

Our study reports the effect of constant temperature storage, intermittent warming, and intermittent CO₂ treatments on 'Primofiori' lemon storage behavior. Correlations between fruit susceptibility to decay and CI and physicochemical characteristics and storage conditions also were investigated.

'Primofiori' lemons were harvested from a 20-year-old orchard on 17 Jan. 1988 in Crevillente (Alicante); Spain, and transported by car the same day to our laboratory in Murcia,

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Table 1. Lemon juice composition at harvest and after 8 weeks of storage under various conditions.

Treatment ^y	Chemical constituents						
	Extractable juice ^x	SSC ^z (% juice)	pH	Acidity (% juice)	Ascorbic acid (mg/100 ml juice)	Reducing sugars (% juice)	Nonreducing sugars (% juice)
At harvest	44.5 c ^w	9.1 a-c	2.3 b	6.7 c-e	45.9 c	1.4 de	1.1 c
Cold storage							
A (2C)	45.3 c	8.6 c	2.3 b	6.6 de	49.2 a-c	1.2 e	0.8 e
B (8C)	49.2 ab	8.9 a-c	2.4 ab	6.2 g	49.5 a-c	1.5 de	0.9 d
C (13C)	49.5 a	9.2 a-c	2.3 b	6.3 fg	49.0 a-c	1.5 de	1.0 d
Intermittent warming							
D	47.6 a-c	8.8 bc	2.3 b	6.9 a-c	47.9 a-c	1.2 e	0.9 d
E	46.3 bc	9.3 ab	2.3 b	7.0 ab	50.1 a-c	1.7 cd	1.0 d
F	49.9 a	9.4 a	2.4 ab	6.7 c-e	52.4 a	1.5 de	0.9 d
G	47.6 a-c	8.6 c	2.5 a	6.5 ef	50.1 abc	1.4 de	1.0 d
H	46.4 bc	9.2 a-c	2.4 ab	7.1 a	49.5 a-c	1.7 cd	1.1 c
CO ₂ for 24 h/week at 13C							
I (10%)	47.6 a-c	9.0 a-c	2.3 b	6.8 b-d	51.3 ab	3.4 a	1.4 a
J (20%)	48.9 ab	8.9 a-c	2.4 ab	6.6 de	47.9 a-c	2.3 b	1.2 b
K (30%)	46.4 bc	8.9 a-c	2.5 a	6.8 b-d	47.4 bc	2.0 bc	1.1 c

^xSSC = soluble solids concentration.^yD = 2 cycles, 1 week 2C, 3 weeks 13C; E = 2 cycles, 1 week 8C, 3 weeks 13C; F = 2 cycles, 2 weeks 2C, 2 weeks 13C; G = 2 cycles, 3 weeks 2C, 1 week 13C; H = 2 cycles, 3 weeks 8C, 1 week 13C.^zPercentage (w/w) of juice to fresh weight.^wMean separation in columns by Duncan's multiple range test, $P \leq 0.05$.

Spain (40 km from Crevillente), where they were kept at 13C. The next day, the fruit were washed with water and dipped in thiabendazole at 2 g-liter⁻¹ at 20C for 3 min. Fruit of uniform size and appearance (120 g, 60 mm in diameter, green-yellow, and free of defects) were randomized into 150-fruit lots as three replicates of 50 fruit each per treatment. The plastic boxes containing the fruit were placed inside 360-liter gas-tight chambers in cold storage rooms at 2, 8, or 13C with an RH >95% in each. The respective vapor pressure differences were ≤ 30 , 60, and 80 Pa.

The treatments were A) 8 weeks at 2C, B) 8 weeks at 8C, C) 8 weeks at 13C, D) 1 week at 2C + 3 weeks at 13C + 1 week at 2C + 3 weeks at 13C, E) 1 week at 8C + 3 weeks at 13C + 1 week at 8C + 3 weeks at 13C, F) 2 weeks at 2C + 2 weeks at 13C + 2 weeks at 2C + 2 weeks at 13C, G) 3 weeks at 2C + 1 week at 13C + 3 weeks at 2C + 1 week at 13C, H) 3 weeks at 8C + 1 week at 13C + 3 weeks at 8C + 1 week at 13C, I) 10% CO₂ for 24 h weekly during storage at 13C, J) 20% CO₂ for 24 h

weekly during storage at 13C, and K) 30% CO₂ for 24 h weekly during storage at 13C.

Physical and chemical analyses were performed on three lo-fruit samples at harvest and after each storage trial. Fruit were weighed with a precision of ± 0.01 g and weight loss was calculated as the percentage of initial fresh weight. Extractable juice obtained by squeezing was calculated as the percentage (w/w) of juice to fresh weight. Juice was used to determine total soluble solids concentration (SSC), pH, titratable acidity (TA), reducing and nonreducing sugars, and ascorbic acid. SSC was determined with an Abbé refractometer (readings at 20C). TA, calculated as the percentage of citric acid, was determined by titrating 1 ml of juice with 0.1 N NaOH. Reducing and nonreducing sugars were determined using the method of Ting (1956). Ascorbic acid was determined by the Association of Official Analytical Chemists (AOAC, 1984) calorimetric method.

Carbon dioxide concentrations were obtained using calibrated capillaries, differential

manometers, and gas mixers enriched with a known flow of compressed air. The gas flow was humidified in a mixer before entering the gas-tight chambers containing the fruit.

After they were removed from storage, all fruit were examined for decay and disorders.

Data were subjected to analysis of variance and means were separated by Duncan's multiple range test, where appropriate.

Juice composition of lemons stored for 8 weeks at 2C did not show any significant difference in constituents, except for a slightly lower nonreducing sugar content compared to fruit at harvest (Table 1). TA and nonreducing sugars decreased in lemons stored at 8 or 13C compared to fruit at harvest. No significant differences were evident between the latter two temperatures. Chemical composition of juice from lemons under intermittent warming was not different or only slightly different from fruit at harvest. Carbon dioxide promoted higher levels of reducing sugars, but nonreducing sugars were higher only in lemons treated with 10% and 20% CO₂ relative to

Table 2. Incidence of weight loss, decay, and physiological disorders of lemons after 8 weeks of storage under various conditions.

Treatment ^y	Wt loss (%)	Decay and disorders (% fruit affected)					
		<i>Alternaria</i>	Red blotch	Peteca	Membranosis	Pitting	Oleocellosis
Cold storage							
A (2C)	2.5 ab ^y	0 c	0 d	0 b	32.6 c	4.0 a	31.3 a
B (8C)	3.4 ab	0 c	9.3 c	0 b	19.3 a	0.7 b	0.7 b
C (13C)	4.8 a	3.3 a	44.0 b	0.7 b	1.3 e	0 b	0 b
Intermittent warming							
D	3.1 ab	2.0 ab	35.3 b	0 b	12.7 de	2.7 b	4.0 b
E	4.4 a	0.7 bc	44.0 b	0 b	51.9 b	0 b	2.7 b
F	4.1 ab	0 c	1.3 c	0 b	25.3 cd	0 b	0 b
G	1.4 b	0 c	0 d	4.0 a	52.6 b	0.7 b	3.3 b
H	1.6 b	0 c	9.3 c	1.3 b	81.0 a	2.7 b	4.0 b
CO ₂ at 13C							
I (10%)	3.1 ab	2.7 a	70.1 a	0 b	0 e	0 b	0 b
J (20%)	1.6 b	2.7 a	73.3 a	0 b	0 e	0 b	0 b
K (30%)	3.3 ab	1.3 a-c	84.0 a	0 b	0 e	0 b	0 b

^yD = 2 cycles, 1 week 2C, 3 weeks 13C; E = 2 cycles, 1 week 8C, 3 weeks 13C; F = 2 cycles, 2 weeks 2C, 2 weeks 13C; G = 2 cycles, 3 weeks 2C, 1 week 13C; H = 2 cycles, 3 weeks 8C, 1 week 13C.^wMean separation in columns by Duncan's multiple range test, $P \leq 0.05$.

fruit at harvest or to most storage treatments (Table 1).

Compared to its value at harvest, ascorbic acid concentration was higher with 2 weeks at 2C + 2 weeks at 13C and 10% CO₂ (Table 1).

Continuous storage at 2C and intermittent warming of two cycles of 2 weeks at 2C + 2 weeks at 13C and two cycles of 3 weeks at 2C + 1 week at 13C were best for retaining original color (data not shown). Few differences in weight loss were evident among the treatments after removing fruit from storage (Table 2).

Thiabendazole was effective in controlling fungal diseases, and incidence of alternaria rot was low (<3%) for all treatments (Table 2). Red blotch, however, was extensive in most treatments, except for storage at 2C and intermittent warming 3 weeks at 2C + 1 week at 13C. Incidence was high in treatments C-E and in CO₂-treated fruits. Peteca, like alternaria rot, also was quite low in the stored fruit.

High CO₂ prevented membranosis, oleocellosis, and rind pitting (Table 2). Continuous storage at 13C was better than any other non-CO₂ treatment for reducing membranosis. The worst storage conditions for membranosis were 3 weeks at 8C + 1 week at 13C and continuous storage at 8C. Although this disorder occurred on many fruit, its intensity was slight. The incidence of rind pitting and oleocellosis was low (<4%), except for lemons stored continuously at 2C.

'Primofiori' (Artés et al., 1981; Guillén et al., 1980) and 'Villa-Franka' (Cohen et al., 1983) lemons are sensitive to low temperatures. There is a close correlation between disorder development and storage conditions. In lemons stored continuously at 2, 8, or 13C, incidence of peteca, membranosis, rind pitting, and oleocellosis was higher at the lower storage temperatures (Artés and Escribe, 1988; Artés et al., 1981, 1990; Chalutz et al., 1981; Cohen et al., 1983, 1990). The best temperature for continuous cold storage in air for 'Primofiori' was 13C, which eliminated the development of peteca, membranosis, pitting, and oleocellosis. Similar results were found by Cohen et al. (1983) with 'Villa-Franka' using the same temperatures. In our study, red blotch was more severe at the higher storage temperature, and intermittent warming reduced its incidence. According to our previous observations (Artés and Escribe, 1988), 'Primofiori' lemons were more sensitive to red blotch and membranosis than to peteca, rind pitting, and oleocellosis (Table 2).

The incidence of decay and rind pitting with intermittent warming regimes of two

cycles of 3 weeks at 2C + 1 week at 13C and two cycles of 3 weeks at 8C + 1 week at 13C in our study agreed with the results obtained by Cohen et al. (1983) with 'Villa-Franka' using the same treatments. However, membranosis was not eliminated in our study, and weight loss was higher in their experiments than in ours after 2 months of cold storage.

The effectiveness of intermittent CO₂ treatments during cold storage to reduce some types of disorders (particularly membranosis, oleocellosis, and rind pitting) has not been reported previously for lemons.

Juice content and composition were similar to that reported by us (Artés et al., 1981; Guillén et al., 1980) in the same variety, and by Cohen (1983, 1988, 1990) in 'Villa-Franka' lemons given similar treatments. The slight increase in ascorbic acid concentration we noted also was reported by El-Nabawy et al. (1977) with 'Eureka' lemons stored continuously in air at 14C for 2 months. The reason for this behavior is unknown. Possibly, the biosynthetic route continues its action under these storage conditions.

This study showed that best quality retention in stored 'Primofiori' lemons was obtained with intermittent warming cycles of 2 weeks at 2C + 2 weeks at 13C and RH >95%. This regime, not previously reported for lemon, prevented the development of alternaria rot, peteca, oleocellosis, and rind pitting and reduced the incidence of red blotch. However, membranosis was not effectively controlled. Under this storage condition, SSC, pH, TA, and reducing sugars did not change relative to values at harvest, but ascorbic acid concentration increased.

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