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Influence of Postharvest Vacuum Infiltration with Calcium on Chilling Injury, Firmness and Quality of Lisbon Lemon Fruit

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Abstract: The aim of present study to determine the effect of postharvest vacuum infiltration with $CaCl_2$ on Chilling injury, firmness and quality of Lisbon lemons [*Citrus limon* (L.) Burm.] at the yellow-green state, were treated with $CaCl_2$ solutions up to 7.5% (w/v) by normal vacuum infiltration (NVI, 15°C, -33 kPa, 10 min), or hot vacuum infiltration (HVI, 45°C, -33 kPa, 10 min) before storage at 1.5°C and 85-90% RH for 6 and 12 weeks and 1 additional week at 20°C. Vacuum infiltration maintained firmness best following 6 weeks, but reduction in the Chilling Injury (CI) index observed following 12 weeks of cold storage and additional week at 20°C. By both infiltration regimes, the different concentrations of $CaCl_2$ only affected CI, weight loss and firmness of fruit, but did not alter other parameters. Among the treatments, NVI at 1.5% $CaCl_2$ and hot water infiltration alone (45°C, -33 kPa, 10 min) were the most effective and reduced the severity of CI by 53.2 and 19.3%, respectively. The fruit treated with 7.5% $CaCl_2$ by NVI and ≥ 4.5% $CaCl_2$ by HVI showed significantly lower values of deformation (more firmness) than non-treated ones. Combination of $CaCl_2$ and hot water (HVI) increased the efficiency of $CaCl_2$ in terms of firmness retention, but had no additive effects in reduction of CI. As compared to NVI, HVI increased CI index and the rate of K^+ leakage and decreased total soluble solids and acidity levels of fruit. A significant correlation was also found between CI index and each of other parameters. As CI increased, weight loss and ion leakage increased too, but ascorbic acid and acidity levels decreased. Similar trends were observed as the storage period advanced and CI increased.

Key words: Chilling injury, quality parameters, postharvest treatments, $CaCl_2$, Lisbon lemon

INTRODUCTION

Many citrus cultivars, particularly lemons, are sensitive to Chilling Injury (CI) and may develop peel injury when exposed to cold treatment, which greatly reduces the fruit marketability (Houck *et al*., 1990). It is also known that calcium plays a role in the maintenance of cell stability, particularly membranes, under stress conditions such as postharvest low temperature storage (Roux and Slocum, 1982). Pre-and postharvest calcium applications have been reported to lower the rate of plant senescence, fruit ripening, sensitivity to pathogen and susceptibility to CI in different fruit and vegetables by reducing or delaying cell wall breakdown, maintaining the membrane function and prolonging the capacity for signal transduction (Poovaiah, 1986; Ferguson and Drobak, 1988). In a different trial, Fortune mandarin fruit showed positive effect of the preharvest calcium in reducing CI (Ait-Oubahou *et al*., 2003; El-hilali *et al*., 2003). In Maglino lemons, postharvest calcium

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immersion in solutions of $CaCl_2$ at concentration up to 0.36 M (4.93%) for 25 min, increased firmness, reduced CO_2 production and prevented color change during 40 days of storage at 12°C (Tsantili *et al*., 2002). Postharvest heating is a non contaminating physical treatment that delays the ripening process, reduces the sensitivity of the produce to low temperatures and controls the activity of parasitic agents. In this way, this technique prolongs the storage and shelf life of the fruits while maintaining their quality (Lurie, 1998). Numerous authors have obtained good results using hot water dipping with lemon fruits as a preventative treatment for CI (Rodov *et al*., 1995; McLauchlan *et al*., 1997). The heat treatment can be complemented with the use of Ca^{2+}. Heat allows demethylation of pectin by pectin methylesterase (PME), to form anionic Coo^- group for Ca^{2+} to form salt bridge cross-links with. This may make the cell wall less accessible to enzymes occurring in the fruit (which cause softening) or to enzymes produced by fungal pathogens (which cause decay) (Conway and Sams, 1987; Sams *et al*., 1993). Immersing apples in water at 45°C reduced *Gloeosporium* decay but increased tissue breakdown. However, adding $CaCl_2$ to the hot water controlled tissue breakdown and fungi (Sharpless and Johnson, 1976).

So far, studies on the CI of citrus fruit have mainly been dealt with the effect of pre-harvest Ca; there were no studies that we could find in the literature deal with postharvest Ca treatment on the CI of lemon fruit. In the present study, the following questions are answered: 1) could vacuum infiltration with $CaCl_2$ at normal and hot temperature reduces CI and firmness loss of lemon fruit following storage at chilling temperature? 2) Which concentration of $CaCl_2$ would be effective? 3) Could combination of heat and Ca applications have additives effects?

The effects of these treatments on the physiological and chemical changes taking place after storage were also measured.

MATERIALS AND METHODS

Source of Fruit, Ca Treatments and Storage Conditions

Lemons [*Citrus limon* (L.) Burm. cv. Lisbon] were harvested at the yellow-green state from 20 year old trees on 6 Dec. 2005 in HajiAbad, south of Iran and transported by car the same day to the laboratory in Shiraz where they kept at 13°C. Within 2 days after harvest, fruit of uniform size and appearance were randomized into two treatment lots of 840 fruits for the following treatments: (1) Normal Vacuum Infiltration (NVI) and (2) Hot Vacuum Infiltration (HVI). Each treatment lot was then divided into seven equal sub groups (120 fruits) and were treated in one of the following ways: No treatment, distilled water, 1.5, 3, 4.5, 6 and 7.5% solution (w/v) of $CaCl_2$ ($CaCl_2$, $2H_2O$) in distilled water. Tween 20 (0.2%) was added to all solutions to improve absorption of the $CaCl_2$. To perform normal vacuum infiltration, fruits were treated with $CaCl_2$ at reduced pressure (-33 kPa; Joyce *et al*., 2001). The calcium solutions were applied in a 15 L capacity vacuum desiccator (model B-1834, Gallenkamp, England) at 15°C. The desiccator was connected to Speedivac high vacuum pump and a negative pressure gauge to maintain and monitor the reduced pressure, respectively. For infiltration, fruits were placed into desiccator and kept submerged for 0.5 min. The vacuum (-33 kPa) headspace over the solution was then drawn for 1.5 min. Upon restoration of atmospheric pressure, fruits were left submerged for further 8 min. To perform hot vacuum infiltration, all steps of normal vacuum infiltration were employed, except the temperature of calcium solutions which was maintained at 45±1°C. Following treatment, fruits were rinsed by dipping for ≈2 sec in distilled water at 15±1°C before drying. Fruits of each treatment (120 fruits) were then divided into 20-fruit lots and placed in netting nylon bags. Each treatment comprised three replicate bags per each of storage period. The bags of fruit were moved to storage room and kept for 6 and 12 weeks at 1.5±0.2°C and 85-90% Relative Humidity (RH), with a complete change of air in the cold room. At the end of storage period the fruits were maintained at 20°C and about 80% RH for 1 week to Simulate a Marketing Period (SMP). All analyses were performed after storage and SMP.

Weight loss and Chilling Injury (CI)

Fruit weight loss was evaluated by weighing the fruit before and after the storage. CI was classified as either peel pitting or surface browning, but these symptoms were combined as a total CI score. CI was rated as 0 (no damage), 1 (slight), 2 (moderate) and 3 (severe). CI index was determined by summing the product of the number of fruit in each category by the score of each category and then dividing this sum by the total number of fruits assessed.

Firmness

Five fruits from each replication of each treatment were analyzed for fruit firmness determination. Fruit firmness was determined by compression tester (Ben-Yehoshua *et al*., 1983) using a 1.5 kg weight on its longitudinal axis. Full deformation (mm) was measured 15 sec after exerting the force on the fruit. The firmer the fruit, the lower reading.

Ion Leakage

Five fruits per replication were used. Five disks (10 mm in diameter, 3 mm thick) consisting of flavedo and albedo tissue were cut with a cork borer, weighted placed in a 100 mL glass bottle, washed twice with deionized water and then incubated into 50 mL glass bottle with 25 mL of 0.2 M manitol at 20±1°C (Saltveit, 2002). Conductivity of the incubation medium was measured using a conductivity meter (Model 644, Metrohm, Swiss) after 4 h incubation under constant shaking. After readings were taken, the bottles were autoclaved at 120°C for 90 min, cooled to 20°C and final conductivity was measured again for total electrolytes. The K^+ content of the bathing solution was measured using flame photometer (Model PFP7, Jenway, England). Percent leakage of total electrolytes and K^+ was calculated as the ratio of the initial reading to the final reading.

Chemical Analysis of Juice (TSS, AA and TA)

Seven fruits per replication were squeezed in an electric juice extractor with a rotating head to determine juice content. Total Soluble Solids (TSS) was then measured using an ATC-1E ATAGO hand-held refractometer on translucent part of the juice after decantation. Ascorbic Acid (AA) was determined by the indophenol titration method (Association of Official Analytical Chemist, 1980). Titratable Acidity (TA) was determined by titration an aliquot of juice against 0.1 N NaOH and expressing the result as percentage of anhydrous citric acid.

Data Analysis

Data were processed for analysis of variance by means of the software SAS system. A split-split-plot design was used, where storage period was the main plot, infiltration regime was the subplot and the concentration of $CaCl_2$ was the sub-subplot. Mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$. Some of the data were also analyzed by simple linear regression analyses.

RESULTS

Chilling Injury

By the end of SMP, storage for 6 weeks resulted in relatively little CI. By both infiltration regimes, no significant differences were observed among the concentrations of $CaCl_2$ on the CI index. In contrast, storage for 12 weeks resulted in significant CI. By NVI regime, all of the $CaCl_2$ concentrations reduced the severity of CI in comparison to untreated and control fruit (Fig. 1 NVI). Among the various $CaCl_2$ concentrations, 1.5% CaCl was the most effective in reducing the development of CI symptoms. This treatment reduced the CI of the fruit by 53.2% as compared with

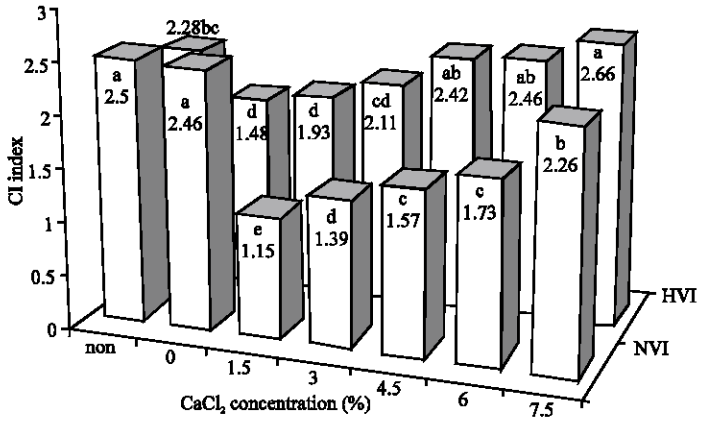

Fig. 1: Effect of $CaCl_2$ concentration on CI of Lisbon lemon, as influenced by infiltration regimes of $CaCl_2$. Fruit stored for 12 weeks at 1.5°C with 85-90% RH and 1 additional week at 20°C. Infiltration regimes: NVI = normal vacuum infiltration, 15°C, -33 kPa, 10 min; HVI = hot vacuum infiltration, 45°C, -33 kPa, 10 min. Values within each column grouping followed by the same letter are not significantly different by DMRT, $p \leq 0.05$

Table 1: Effects of infiltration regime of $CaCl_2$ and storage period on the external and internal quality of Lisbon lemon

		Infiltration regime[w]					
		NVI			HVI		
		Storage period (weeks)[x]			Storage period (weeks)		
Quality	Parameters	6	12	Mean	6	12	Mean
External	Chilling Injury index	0.7a[y]	1.8b	1.3A[z]	0.8a	2.2b	1.5B
	Weight loss (%)	8.5a	16.0b	12.2A	7.4a	16.3b	11.8A
	Electrolyte leakage (%)	56.8a	67.7b	62.2A	59.8a	70.1b	64.9A
	K^+ leakage (%)	53.3a	75.0b	64.1A	56.5a	80.9b	68.7B
Internal	Ascorbic Acid (mg/100 cc)	68.6a	56.4b	62.5A	66.6a	51.4b	59.0A
	Titratable Acidity (%)	7.3a	6.3b	6.8A	6.9a	6.0b	6.4B
	Total Soluble Solids (%)	8.9a	8.9a	8.9A	8.6a	8.6a	8.6B

[w]The details of infiltration regimes were described as in Fig. 1. [x]Fruits stored for 6 and 12 weeks at 1.5°C with 85-90% RH and 1 additional week at 20°C. [y]Values within each row grouping followed by the same lower case letter (a, b) are not significantly different by DMRT, $p \leq 0.05$. [z]Between infiltration regime, values within each row followed by the same capital letter (A, B) are not significantly different by DMRT, $p \leq 0.05$

control fruit. The results showed that as the $CaCl_2$ concentration increased, the effectiveness of Ca in reduction of CI became more negative. By HVI regime, fruit vacuum infiltred with distilled water at 45°C for 10 min showed significant lower CI than untreated fruit (19.3%) (Fig. 1 HVI). Hot $CaCl_2$ concentrations ≥ 4.5% increased CI as compared with untreated and hot water treated fruit. The results showed that the addition of $CaCl_2$ into hot water was ineffective in reducing CI. CI index was also affected by infiltration regime of $CaCl_2$. Susceptibility to CI was significantly greater in fruit treated by HVI than NVI (Table 1).

Weight Loss and Ion Leakage

By both infiltration regimes, weight loss following 6 weeks and ion leakage following 6 and 12 weeks after storage were not influenced by concentration of $CaCl_2$. However, after 12 weeks the fruit treated with 6 and 7.5% $CaCl_2$ showed significantly more weight loss than the other fruit (Table 2). Moreover, K^+ leakage was influenced by infiltration regime of $CaCl_2$. As compared to NVI,

Table 2: Effect of $CaCl_2$ concentration on weight loss (%) of Lisbon lemon after storage for 6 and 12 weeks at 1.5°C with 85-90% RH and 1 additional week at 20°C, as influenced by infiltration regimes of $CaCl_2$

	Weight loss (%)			
	NVI[x]		HVI	
	Storage period (weeks)		Storage period (weeks)	
$CaCl_2$ (%)	6	12	6	12
Non	7.8a[y]	14.5c	7.9a	15.5b
0	8.5a	14.6c	6.8a	14.8b
1.5	7.8a	15.2bc	7.7a	15.3b
3	8.6a	15.7abc	6.9a	15.5b
4.5	9.1a	16.0abc	7.5a	16.0ab
6	9.1a	17.7ab	7.0a	18.2a
7.5	8.1a	18.2a	7.7a	18.9a
Mean	8.4A[z]	16.B	7.3A	16.3B

[x]The details of infiltration regimes were described as in Fig. 1, [y]Values within columns followed by the same lower case letter (a, b, c) are not significantly different by DMRT, $p \leq 0.05$, [z]Values within row followed by the same capital letter (A, B) are not significantly different by DMRT, $p \leq 0.05$

Table 3: Effect of $CaCl_2$ concentration on firmness (expressed as mm deformation) of Lisbon lemon after storage for 6 and 12 weeks at 1.5°C with 85-90% RH and 1 additional week at 20°C, as influenced by infiltration regimes of $CaCl_2$

	Firmness (mm deformation)			
	NVI[x]		HVI	
	Storage period (weeks)		Storage period (weeks)	
$CaCl_2$ (%)	6	12	6	12
Non	2.2ab[y]	3.6b	2.1a	3.6bc
0	2.4a	3.5c	2.1a	3.4c
1.5	2.2ab	3.5c	2.1a	3.5c
3	2.0cab	3.4c	1.9ab	3.6bc
4.5	1.9cab	3.6ab	1.6b	3.4c
6	1.7cb	3.6ab	1.6b	3.7ab
7.5	1.5c	3.7a	1.5b	3.8a
Mean	2.B[z]	3.6A	1.9B	3.5A

[x]The details of infiltration regimes were described as in Fig. 1. [y]Values within columns followed by the same lower case letter (a, b, c) are not significantly different by DMRT, $p \leq 0.05$. [z]Values within row followed by the same capital letter (A, B) are not significantly different by DMRT, $p \leq 0.05$

HVI increased the rate of K^+ leakage of fruit (Table 1). The correlation coefficient from simple linear regression analyses between CI index and weight loss, K^+ and electrolyte leakage were 0.892, 0.826 and 0.644, respectively (p = 0.1%). Differences in weight loss and ion leakage became more apparent as period of cold storage progressed from 6 to 12 weeks (Table 1).

Firmness

Significant difference in preventing softening appeared between control and Ca treated fruit only after 6 weeks of cold storage. The fruits that treated with 7.5% $CaCl_2$ by NVI and those treated with ≥4.5% $CaCl_2$ by HVI showed significantly lower values of deformation than non- treated ones (Table 3). Moreover, there was a negative linear relationship between $CaCl_2$ concentration and fruit deformation (p = 1%). The correlation coefficient from simple linear regression analyses between the $CaCl_2$ concentration and deformation of fruits treated by NVI and HVI were -0.69 and -0.74, respectively. Following 12 weeks of cold storage and SMP, the fruit became softer and no beneficial effects of calcium were observed between firmness of Ca treated and that of non-Ca treated fruit.

Juice Characteristics

By both infiltration regimes, following 6 and 12 weeks of cold storage and SMP, there were no differences among the concentrations of $CaCl_2$ on the internal quality attributes of fruits. Internal quality characteristics influenced by infiltration regimes of $CaCl_2$. As compared to NVI, HVI decreased TSS and TA levels of fruit. HVI also decreased AA levels of fruit, but this effect was not significant (Table 1). As period of cold storage progressed from 6 to 12 weeks, the levels of AA and TA significantly decreased, but TSS was not influenced (Table 1). In general, a significant correlation ($p = 0.1\%$) was found between CI index and AA and TA. As CI increased, AA ($r = -0.846$) and TA ($r = -0.73$) decreased.

DISCUSSION

Ca treatments were shown to be ineffective in significantly reducing CI after 6 weeks of cold storage. This result is thought to reflect the comparatively low incidence of CI rather than the lack of effectiveness of Ca treatments. The severity of the injury is related to the storage temperature and duration (Cohen and Schiffman-Nadel, 1978) and the stage of maturity of the fruit at harvest (Houck *et al*., 1990). The shorter the storage period and the more mature the fruit the lower is the incidence of CI. Present results are consistent with these conclusions, since harvest date was between early October and late January and shorter storage period resulted in little CI.

In other experiments with 'Fortune' mandarin, pre-harvest Ca reduced the level of CI (Zaragoza *et al*., 1997; El-hilali *et al*., 2003). Ait-Oubahou *et al*. (2003) reported a reduction of 40-60% of CI with a different formulation containing Ca, but with weight losses significantly higher than the control. In the present study, we found that postharvest Ca infiltration significantly reduced CI development of lemon fruit and the treatment with 1.5% $CaCl_2$ being the most effective of the treatments, without augmenting fruit weight loss. Our finding regarding the effect of postharvest Ca treatment in reducing CI in lemon are consistent with those of recent study of this treatment on Fortune mandarin (D'Aquino *et al*., 2005).

Infiltration of hot water alone significantly reduced CI of lemon fruit. The beneficial effect of hot water treatment to reduce the sensitivity of citrus fruit to CI, including lemon, has been shown in different studies (Rodov *et al*., 1995; McLauchlan *et al*., 1997). However, hot $CaCl_2$ solutions had no advantage compared with hot water alone and at higher concentrations increased CI and weight loss of fruit. Accordingly, as compared to NVI, HVI enhanced the CI and ion leakage of fruit. The unsatisfactory results from combination of heat and $CaCl_2$ may be associated with cellular breakdown, loss of membrane integrity (as measured by ion leakage) and by the apparent removal of epicuticular waxes, which are known to play and important role in water exchange through the rind (El-otmani *et al*., 1989). This agrees with findings in citrus fruit that the rate of water loss during low storage temperature is to be contributing factor to CI development (Purvis, 1984). Since, NVI with the same concentrations of $CaCl_2$ was effective and reduced the severity of CI, suggest that Ca concentration range applied at HVI condition was not optimal and modified procedures may be needed to achieve additive effect of these treatments.

Another hypothesis raised by present data concerns the effects of concentration of Ca^{2+} ion on biochemical responses of lemon fruits. The mechanism, by which calcium may cause the physiological changes, resulted in decreasing or increasing CI is not clear at present, but some related suggestions may confirm our results. It has been known that the maintenance of low level of cytoplasmic calcium is required for the start of the function of the calcium messenger system. The transient increase in Ca^{2+} levels in chilling-insensitive plants is suggested to act as a second messenger in low temperature signal transduction during cold acclimation (Monroy and Dhindsa, 1995). Under chilling, the prolonged high level of intracellular Ca^{2+} in chilling-sensitive plants here defined as Ca^{2+} overload, is thought to be cytotoxic and has been proposed to act as physiological transducer of cell injury or death (Minorsky, 1985).

Present results have shown that treatment with Ca could be effective in terms of retention firmness in a shorter period of cold storage. The lower CI and weight loss during 6 weeks of cold storage, would suggest that Ca treatment donated more degree benefit. All $CaCl_2$ concentrations tested from 1.5 to 7.5% were effective but optimum concentration for firmness retention achieved at higher levels and affected by infiltration regimes of $CaCl_2$. Similar effects have been found in yellow-green Maglino lemons that were immersed in $CaCl_2$ solution at concentrations up to 0.36 M (4.93%) for 25 min and stored at 11°C for 40 day, except 0.09 M $CaCl_2$ was optimum (Tsantili *et al*., 2002). The use of exogenous Ca during postharvest of fruit to maintain the texture has been also reported in 'Verna' lemon (Valero *et al*., 1998) and apple (Wang *et al*., 1993). The calcium firming effect has been attributed to their cross linking to pectic substances in the cell wall, resulting in the formation of calcium pectate that is detectable immediately after treatment (Conway *et al*., 1997). This binding blocks also the access of degradative enzyme to the cell wall reducing the rate of softening during storage (Conway and Sams, 1987). Firmness of citrus fruit depends primarily on turgidity and on the weight loss rate (Ben-Yehoshua *et al*., 1983). However, in the present work it was noted that improvement of fruit firmness by Ca treatments was not accompanied by additional weight loss reduction as compared with non-treated fruit. In this case, Ca may have affected fruit firmness either by inhibiting enzymatic systems involved in softening, or by elicitation of cell wall strengthening processes.

The firmness data comparing HVI-treated and NVI-treated fruits indicates that lower concentrations of $CaCl_2$ needed for better maintain firmness when fruit infiltred at 45°C (HVI). Indeed, heat increased the efficiency of calcium. This demonstrates that in lemons, postharvest treatments, heat and Ca may act synergistically to maintain or even enhance the initial firmness values of the fruits. Similar effect has been found in apples (Sams *et al*., 1993; Klein and Lurie, 1994) and strawberries (Garcia *et al*., 1996). The combination of both treatments probably allows the formation of salt bridge cross links with the pectin molecules of the cell wall after their heat-induced demethylation (Sams *et al*., 1993).

In addition to firmness retention, Ca treatments further alleviated CI, without affecting AA, TSS and acidity levels. Similar findings of retention firmness of the fruit without alteration of their internal quality parameters have been reported previously in Verna (Valero *et al*., 1998) and Maglino (Tsantili *et al*., 2002) lemons. Concerning decrease the internal quality attributes of lemons after prolonged cold storage as well as by HVI, could be explained by a greater of CI. The decrease in TSS and acidity levels also was reported by Artes *et al*. (1993) with Primofiori lemons which stored continuously at 2°C for 8 weeks. There was a close correlation between susceptibility to CI and the respiration, ethylene or volatile content in the internal atmosphere of lemons following chilling temperatures (Eaks, 1980). Possibly, these factors contribute to alter the atmosphere and thus may affect metabolism and that in turn, impact on internal quality.

In conclusion, vacuum infiltration of calcium was effective in reducing CI and firmness loss of lemon fruit following chilling temperature. Furthermore, combination of Ca and heat increased the efficiency of Ca in terms of retention firmness, but had no additive effects in reduction CI. Ca treatment seems to be a promising method to improve quality of lemons without affecting juice characteristics. However, to apply it commercially and to obtain optimum effect of Ca treatment, the appropriate procedure and treatment conditions need further investigation.

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