Influence of Postharvest Treatment with Putrescine and Calcium on Endogenous Polyamines, Firmness, and Abscisic Acid in Lemon (*Citrus lemon* L. Burm Cv. Verna)

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Fruit firmness, free polyamine levels (putrescine, spermidine, and spermine), abscisic acid (ABA), and color index were determined in lemon fruit at two ripening stages (at color break and fully yellow) after vacuum infiltration with 1mM putrescine and 1 mM calcium chloride. Both treatments significantly increased fruit firmness as compared with control fruit in stage 1 lemons. Putrescine-treated fruits showed higher levels of firmness and lower weight loss than calcium-treated or nontreated fruits during storage. The concentrations of putrescine, spermidine, and spermine were higher in stage 1 than in stage 2 lemons, whereas the opposite was found for ABA. The treatment with putrescine was the most effective to maintain higher levels of endogenous putrescine and spermidine, but only for stage 2 fruit. Both putrescine and calcium treatments delayed the color change, which was related to the lowest levels of ABA found.

Keywords: *Lemon; Citrus lemon; polyamines; abscisic acid; postharvest; firmness; color*

INTRODUCTION

In *Citrus* species, fruit growth and development are complex processes that must be coordinated in time, and their studies have included the changes in endogenous levels of one or more plant hormones, such as polyamines and abscisic acid (ABA) (El-Otmani et al., 1995). Polyamines occur widely in plants and have been reported to have regulatory effects related to cell division and growth (Smith, 1985). Polyamines are synthesized from ornithine and arginine via ornithine decarboxylase (ODC) and arginine decarboxylase (ADC). ODC has been linked to the cell cycle and rapid cell division, whereas ADC has been linked to stress responses (Evans and Malmberg, 1989). Putrescine is essential for tomato fruit development, and ODC is the enzyme responsible for the synthesis of putrescine and the application of putrescine depressed ODC in the absence of inhibitors, thus suggesting a feedback control for this enzyme (Cohen et al., 1982). A relationship between putrescine-enhanced level and the delay in the senescence process during the ripening of long-keeping tomato fruit has been described (Dibble et al., 1988).

The presence of ABA in *Citrus* fruit during ripening is well documented, and several authors suggest that ABA plays an essential role in fruit development and that changes in the endogenous levels are directly related to the ripening process (Goldschmidt, 1976; Nooden, 1988; Valero et al., 1992). ABA may be involved in fruit color development, since the content of ABA in flavedo increases throughout the process of *Citrus* fruit development (Aung et al., 1991). Suresh et al. (1978) showed a relationship between ABA and polyamines, because ABA decreased putrescine and ADC activity. However, ABA did not seem to affect polyamine levels or their rates of change during avocado ripening (Cutting et al., 1990). Little is known about endogenous levels of ABA during the postharvest physiology of fruits; however, some controversial results have been reported.

Information about the role of the endogenous polyamine levels for *Citrus* is limited, and most of the work has focused on the study of chilling injury (CI). Cold storage of mandarin, grapefruit, and orange fruits increases the level of putrescine, and the magnitude of this response was different in each cultivar (Yuen et al., 1995). ODC and ADC had the greatest activities in fully developed but unripe mandarin and were accompanied by increases in putrescine and, less significantly, spermidine (Nathan et al., 1984). Increased putrescine levels during the ripening of orange have also been reported (Hasdai et al., 1986).

Since polyamines are implicated in fruit development and ripening and may also influence the postharvest physiology of fruits (Kramer and Wang, 1989), there are some reports on the influence of treatments with several polyamines, such as putrescine, spermidine, and spermine, and their role in fruit physiology. Exogenous applications of polyamines retarded softening of apples and strawberries during storage (Kramer et al., 1991; Ponappa et al., 1993). On the other hand, the importance of Ca^{2+} in maintaining the postharvest quality of fruits is well documented. Pressure infiltration with calcium chloride (Ca) solution at harvest gave firmer fruits and less breakdown in the cell wall due to the ability of Ca^{2+} to bind free carboxyl groups, but an excess of Ca^{2+} remained in the intercellular spaces caused on negative fruit injury (Conway et al., 1995). As the mechanism of Ca^{2+} action appears to involve its

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cationic nature, polyamines are also positively charged and have properties similar to those of calcium in terms of their ability to delay senescence and retard fruit softening (Wang et al., 1993).

In this paper the effect of exogenous postharvest treatment with putrescine and calcium chloride on polyamine and ABA levels in lemon at two ripening stages, at a color break, and fully ripe colored fruit was studied. Also, the possible roles of polyamines and ABA in fruit firmness and color development in retarding the senescence process during storage at 15 °C have been investigated.

MATERIALS AND METHODS

Plant Material. Lemon (*Citrus lemon* L. Burm cv. Verna) fruit was harvested from Tres Caminos-CEBAS-CSIC experimental farm in Murcia (Spain) at two stages of ripeness: stage 1 (at color break) and stage 2 (mature yellow color). Some of the characteristics of the fruits were the following: (stage 1) weight = 168.4 ± 1.8 g, diameter = 67.0 ± 1.4 mm, color (*a*/*b* parameter) = −0.32 ± 0.01, total soluble solids (SSC) = 7.38 ± 0.19, titratable acidity (TA) 5.24 ± 0.24; (stage 2) weight = 164.6 ± 0.9 g, diameter = 63.0 ± 0.6 mm, color (*a*/*b* parameter) = −0.10 ± 0.01, SSC = 6.80 ± 0.11, and TA = 4.62 ± 0.20.

For each ripening stage 200 fruits were picked from mature trees. Once in the laboratory 65 lemons were selected in accordance with their color and weight. They were randomized and divided into 3 lots of 20 fruits for the following treatments: (1) control (distilled water); (2) 1 mM putrescine; and (3) 1 mM calcium chloride. Tween 20 (0.2%) was added to all solutions to improve the absorption of the chemicals. The treatments were performed by vacuum infiltration by placing the fruits in 5 L of solution and applying 200 mmHg for 8 min. Following infiltration the fruits were placed on Kraft paper and allowed to dry before storage at 15 °C in a temperature-controlled chamber, in permanent darkness and with a relative humidity of 90%. After 1, 7, 14, and 21 days, five fruits for each treatment were sampled. They were weighed and the color was measured. For each fruit 10 individual disks (flavedo plus albedo) of 10 mm diameter were sampled, which altogether were immediately frozen in liquid N_2, then milled to obtain an homogeneous sample, and stored at −20 °C until polyamines and ABA were analyzed. Five nontreated fruits were sampled to determine the initial stage after picking, in which the same procedures and determinations were made as above (day 0).

Color Determination. Color was determined using the Hunter Lab System in a Minolta colorimeter model CR200. The ratio between the parameters *a* and *b*, expressed as (*a*/*b*), was taken into account by means of three determinations for each fruit along the equatorial axe.

Firmness Determination. Magness−Taylor firmness was recorded using a 5 mm probe mounted on a Universal Assay Machine Lloyd, model LR5K (Lloyd Instruments) interfaced to a personal computer. Two measurements were made on two equatorial fruit zones at 90°. A beveled holder prevented bruising of the opposite side. The machine was prepared to travel at 20 mm min^{-1} for 10 mm after contacting the skin. The machine determined the maximum force of the skin penetration (flavedo plus albedo), and the results were expressed in newtons.

Total SCC and TA Determination. TA was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1 using 1 mL of diluted juice in 25 mL of distilled H_2O. The results were expressed as grams of citric acid per 100 g of fresh weight. Two measurements were made from each fruit. Total SSC was determined by a P20 RL2 refractometer at 20 °C. Three determinations were made from each fruit.

Polyamine Analysis. Free polyamines were analyzed according to the method of Flores and Galston (1982). Two extractions of polyamines were made from each lemon. One gram of fresh tissue was extracted with 10 mL of 5% cold perchloric acid. 1,6-Hexanediamine (100 nmol g^{-1}) was added as internal standard. The homogenate was then centrifuged for 30 min at 20000*g*. Free polyamines left in the supernatant were benzoylated as previously described (Serrano et al., 1995). Derivatives were analyzed by HPLC. The elution system consisted of MeOH/H_2O (64:36) solvent, running isocratically with a flow rate of 0.8 mL min^{-1}. The benzoylpolyamines were eluted through a reversed-phase column (LiChroCart 250-4,5 μm) and detected by absorbance at 254 nm. A relative calibration procedure was used to determine the polyamines in the samples, using 1,6-hexanediamine as the internal standard and standard curves for putrescine, spermidine, and spermine.

ABA Analysis. ABA was extracted from 0.5 g of fresh tissue fruit with 10 mL of a solution of 80% acetone containing butylated hydroxytoluene at 100 mg L^{-1} and citric acid at 0.5 g L^{-1}. The extracts were diluted suitably with a 50 mM Tris buffer, pH 7.8, containing 1 mM $MgCl_2$ and 150 mM NaCl and then quantified by an enzyme-linked immunosorbent assay (ELISA) (Weiler, 1980), using an IgG monoclonal antibody (Idexx, Inc., San Bruno, CA). Synthesis of ABA−bovine serum albumin conjugate was carried out as described by Weiler (1980). ABA content was estimated from the standard curve prepared for each particular plate using an spectrophotometer StarFax 2100 (Awareness Technology Inc.). The absorbance was fixed at 405 nm. Three extractions were made from each sample, and each extract was quantified in duplicate. For each extract, three dilutions were prepared and at least two of them fell on the standard curve. All manipulations were carried out in dim light. The ABA levels were consistent with the appropriate dilution. No interference from impurities was detected when ABA standards were added to diluted extracts of fruits.

Statistical Design. A two-factor ANOVA was made for each ripening stage to determine the effects of treatments and storage time on polyamine, ABA, weight loss, firmness, and color levels. Mean comparisons were performed using the HSD Tukey test. All analyses were performed with SAS statistical software package.

RESULTS

Effect of Putrescine and Ca Treatments on Firmness. The concentration of putrescine was chosen by taking into account experiments in which treatments with levels of putrescine >1 mM resulted in the development of surface damage (Kramer et al., 1991). Magness−Taylor firmness showed significant differences in relation to the treatments applied and storage time (Table 1). Initial values of firmness were 39.35 ± 1.11 and 32.34 ± 2.24 N in stages 1 and 2, respectively (Figure 1). Significant increases in firmness after the treatments and during storage were observed for stage 1 fruit as compared with the nontreated fruit (Table 2). In stage 1 lemons (Figure 1a), firmness values were 41.16 ± 1.24 N for putrescine-treated fruits and 46.09 ± 1.91 N for Ca-infiltrated fruits 1 day after the infiltrations. Firmness for control lemons decreased until 38.8 ± 1.82 N. Firmness level for stage 2 lemons 1 day after the treatments were 35.71 ± 1.26 N for putrescine-treated fruits and 35.03 ± 1.10 N for Ca-treated fruits, whereas levels in control fruits slightly diminished to 31.76 ± 1.62 N (Figure 1b).

These differences in fruit firmness between control and putrescine-treated fruits became greater during storage at 15 °C for 21 days in stage 1 lemon. For instance, after 14 days, the firmness was significantly higher for putrescine-treated fruits (46.35 ± 2.11 N) as compared with control ones (38.01 ± 2.44 N). Ca-treated fruits also showed higher firmness levels than control lemons, but the differences were only significant 1 day after the infiltration (Table 2). With regard to

Table 1. ANOVA for Dependent Variables for Treatments Applied, Storage Times, and Their Interactions for Two Ripening Stages of Lemons[a]

	putrescine	spermidine	spermine	ABA	wt loss	firmness	color
stage 1							
time	***	ns	***	***	***	***	***
treatment	ns	***	*	**	**	*	***
time × treatment	ns	*	***	***	ns	ns	ns
stage 2							
time	***	***	***	***	***	ns	*
treatment	***	**	ns	ns	ns	ns	ns
time × treatment	*	**	ns	***	ns	ns	ns

[a] ***, **, and * represent significance at the 0.001, 0.01, and 0.05 levels, respectively, and ns represents nonsignificance at $P < 0.05$ level.

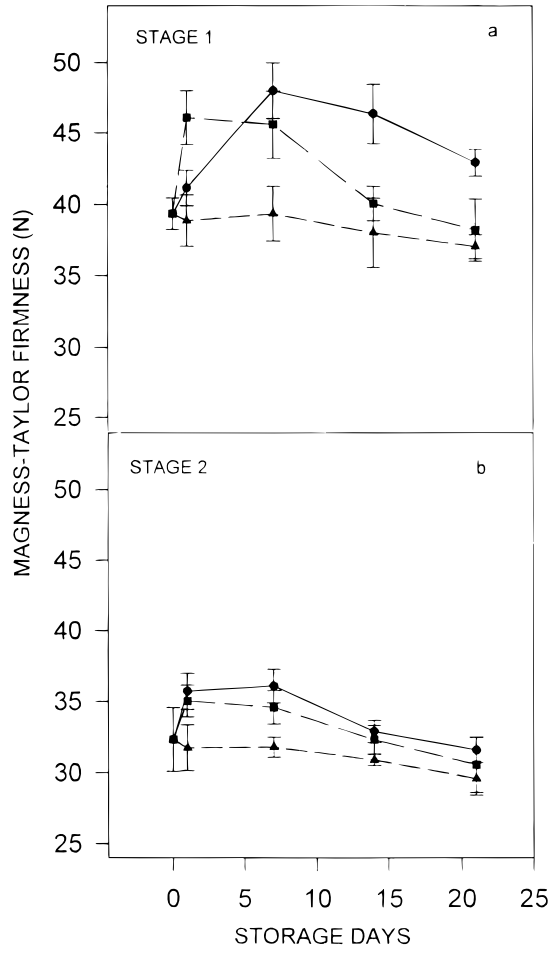

Figure 1. Firmness changes (newtons) in stage 1 (a) and stage 2 (b) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of determinations made in five fruits in duplicate ± SE.

stage 2 lemons, a similar behavior was observed. Putrescine-treated fruits became firmer than calcium-treated ones, and both treated lemons were firmer than control fruits. However, significant differences appeared between control and putrescine-treated fruits only during the first week of storage (Table 2) (Figure 1b). For both ripening stages, the fruit firmness levels at the end of the storage period were higher than the initial ones just for the putrescine-treated lemons.

Color Development, SCC, TA, and Weight Loss. The evolution of lemon color during storage at 15 °C is shown in Figure 2a,b, expressed as the ratio between parameters *a* and *b*. No differences were found in the stage 1 fruits (unripe lemon) 1 day after the infiltrations with putrescine and Ca with respect to control fruits. However, from the second week of storage and until the end of the experiment, both putrescine-treated and Ca-treated fruits delayed the color change to yellow as compared with control fruits, although the differences were significant only for Ca-infiltrated fruits (Table 2). Thus, the initial *a*/*b* parameter (-0.32 ± 0.03) evolved to -0.19 ± 0.01 in putrescine-treated lemons, to -0.22 ± 0.02 for Ca-treated fruits, and to -0.17 ± 0.01 in control ones. The infiltration with Ca was more effective than putrescine treatment to retard the color change in relation to the control lemons

On the other hand, stage 2 fruit (fully yellow) did not show any significant differences with respect to color evolution for either putrescine-treated fruits or Ca-treated fruits as compared with control lemons (Tables 1 and 2). This behavior was expected since at this time of the ripening process, very low changes should appear due to the uniform yellow color of the fruits. Nevertheless, both treatments showed less variation in the evolution of the color with respect to control fruits.

None of the treatments affected the SCC and TA levels during storage of both ripeness stages lemons, since the initial values remained unchanged (data not shown).

In relation to weight loss (Figure 2c,d), just the putrescine-treated stage 1 fruits showed lower weight loss than Ca-treated or control fruits, although the differences were not significant (Table 2). For stage 2 fruits, as happened with color development, no differences were found between the treatments and the control lemons (Table 2).

Polyamine Levels. Free polyamine levels were analyzed in the peel (flavedo plus albedo) of lemon fruits. In both fruit stages, the three major polyamines were quantified: putrescine (Figure 3), spermidine (Figure 4), and spermine (Figure 5). Putrescine and spermidine were the predominant polyamines with the lowest levels of spermine. On 0 day, lemons in stage 1 showed higher concentrations of putrescine, spermidine, and spermine than stage 2 fruits. In stage 1 lemons (Figure 3a), the application of 1 mM putrescine increased the initial level of this amine from 84.6 ± 7.0 nmol g^{-1} of fresh weight immediately after harvesting to 91.9 ± 10.1 nmol g^{-1} of fresh weight 1 day after the vacuum infiltration, but those differences were not significant (Table 2). Ca-treated and control lemons showed similar putrescine concentrations 24 h after treatments to those found in just harvested fruits ($86.9 \pm 8.5^{-}$ and 83.1 ± 1.7 nmol g^{-1} of fresh weight, respectively). Putrescine tended to decline in putrescine-treated fruits during storage with significantly lower levels on the 14th day (43.4 ± 2.6 nmol g^{-1} of fresh weight) as compared with Ca-treated (74.6 ± 6.0 nmol g^{-1} of fresh weight) and control fruits (70.4 ± 3.6 nmol

Table 2. ANOVA for Dependent Variables for Two Ripening Stages of Lemons for Different Treatments and 0, 1, 7, 14, and 21 Days of Storage

treatment	days	putrescine	spermidine	spermine	ABA	wt loss	firmness	color
stage 1								
control	0	a w	a wx	a w	a w	a w	a w	a w
	1	a w	a x	ab x	a w	ab w	a w	a w
	7	a wx	ab x	a x	a x	a w	a w	a w
	14	a wx	a wx	a x	a x	a x	a w	a x
	21	a x	a x	a y	a x	a x	a w	a x
putrescine	0	a w	a w	a w	a w	a w	a w	a w
	1	a w	a w	a y	a w	a w	ab wx	a w
	7	a wx	a w	b wx	a x	a x	b x	a w
	14	b x	b w	a wxy	a x	a y	b wx	ab x
	21	a x	a w	b xy	b x	a z	b wx	ab x
calcium	0	a w	a w	a wx	a wx	a w	a w	a w
	1	a w	a w	b x	a x	b w	b w	a w
	7	a w	b w	a x	a w	a x	ab w	a w
	14	a w	a w	a x	a w	a y	ab w	b x
	21	a x	a w	a w	ab y	a z	ab w	b x
stage 2								
control	0	a wx	a wx	a w	a wx	a w	a w	a w
	1	a w	a xy	a x	a wx	a w	b w	a w
	7	a w	a xy	a x	a x	a w	a w	a w
	14	a wx	a w	a x	a y	a x	a w	a w
	21	a x	a y	a wx	a w	a y	a w	a w
putrescine	0	a w	a wx	a w	a w	a w	a w	a w
	1	a wx	b w	b wx	a w	a w	b w	a w
	7	b x	a w	a x	b x	a w	b w	a w
	14	b x	a w	b x	ab y	a x	a w	a w
	21	b w	a x	a w	b y	a y	a w	a w
calcium	0	a w	a wx	a w	a x	a w	a w	a w
	1	a w	ab w	b wx	a x	a w	ab w	a w
	7	ab w	a wx	a x	b y	a w	a w	a w
	14	ab w	a wx	ab x	b y	a x	a w	a w
	21	b w	a x	a wx	b y	a y	a w	a w

[a] For each ripening stage and storage time, the same letter (a, b, c) among columns is not significantly different among treatments at $P < 0.05$ level. For each ripening stage and treatment, the same letter (w, x, y, z) among columns is not significantly different during storage times at $P < 0.05$ level.

g^{-1} of fresh weight) (Table 2). In contrast, after the putrescine infiltration for stage 2 lemons (Figure 3b), the endogenous levels of this amine increased during the next 7 days and remained without significant change up to the 14th day, reaching then levels of 65.7 $\pm$ 2.8 nmol g^{-1} of fresh weight, with significant differences with respect to Ca-treated (53.9 $\pm$ 6.3 nmol g^{-1} of fresh weight) and control fruits (47.2 $\pm$ 5.0 nmol g^{-1} of fresh weight), to end up decreasing on the 21st (48.8 $\pm$ 7.3 nmol g^{-1} of fresh weight) (Table 2).

In relation to spermidine (Figure 4), a different behavior was also found between stage 1 and 2 lemons. Thus, in putrescine-treated stage 1 fruits (Figure 4a), the initial endogenous spermidine levels (63.1 $\pm$ 15.8 nmol g^{-1} of fresh weight) diminished during the first week of storage (40.8 $\pm$ 8.7 nmol g^{-1} of fresh weight), whereas in stage 2 fruits (Figure 4b) an increase of this polyamine was observed, rising from 45.0 $\pm$ 4.7 nmol g^{-1} of fresh weight on 0 day to 69.8 $\pm$ 4.6 nmol g^{-1} of fresh weight 1 day after the infiltration. The concentration of spermidine in these fruits remained unchanged during the next 14 days until reaching the lowest values on the 21st day (27.0 $\pm$ 1.3 mol g^{-1} of fresh weight).

In Ca-infiltrated fruits the spermidine levels increased for the stage 1 lemons (Figure 4a) during the first week (89.1$\pm$ 9.1 nmol g^{-1} of fresh weight) and slowly decreased until the end of the storage period (71.0 $\pm$ 10.3 nmol g^{-1} of fresh weight). For stage 2 fruits, this increase appeared only on the first day after the treatment (61.9 $\pm$ 12.7 nmol g^{-1} of fresh weight) and then declined throughout the storage process, reaching values of 25.6$\pm$ 2.0 nmol g^{-1} of fresh weight at the end of the experiment.

The initial spermine levels (12.8$\pm$ 4.7 nmol g^{-1} of fresh weight) for stage 1 fruits significantly diminished until the lowest levels after 7 days (Table 2), with the exception of putrescine-treated lemons, which showed a significant increase of spermine on the seventh day (10.2 $\pm$ 1.6 nmol g^{-1} fresh weight) and then a slight decrease at the end of the storage period. In Ca-treated and control fruits, the spermine concentrations experienced a significant increase, reaching the highest levels after 21 days of storage (Table 2) (Figure 5a). For stage 2 fruits (Figure 5b), the levels on day 0 (8.0 $\pm$ 1.6 nmol g^{-1} of fresh weight) dropped during the next 7 days of storage, reaching the lowest concentrations for both treatments, with a final tendency to increase. Control fruits declined their levels of spermine on the first day after storage and remained unchanged until the end of the storage period.

ABA Levels. The contents of ABA for both ripening stages are shown in Figure 6. ABA levels showed significant differences in relation to the treatments applied, storage time, and their interaction (Table 1). Greater concentrations of ABA were found in stage 2 fruits with respect to stage 1 fruits. In stage 1 lemons, all treatments decreased the initial levels of ABA 1 day after the infiltration (6.3 $\pm$ 0.6 nmol g^{-1} of fresh weight). From this time, putrescine-treated and Ca-treated fruits showed similar patterns in the ABA evolution. A 2$-$3-fold increase during the storage period was found, with no significant differences between treatments. In control lemons the increase in ABA levels during the storage period was significantly higher than in putrescine-treated fruits, reaching the maximum concen-

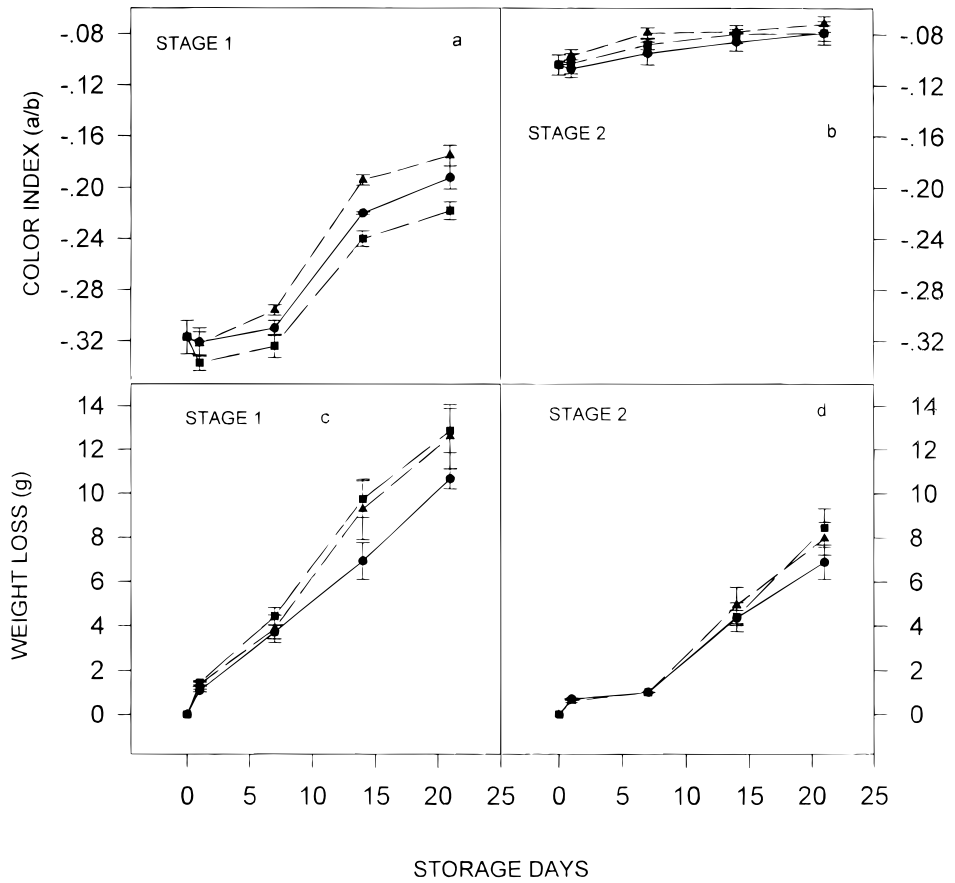

Figure 2. Color index (*a*/*b* parameter) and weight loss (g) in stage 1 (a and c) and stage 2 (b and d) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of determinations made in five fruits in triplicate ± SE.

trations (13.7 ± 0.6 nmol g^{-1} of fresh weight) at the end of the experiment (Table 2) (Figure 6a).

For stage 2 (Figure 6b), the levels on day 0 (13.0 ± 0.4 nmol g^{-1} of fresh weight) diminished during the next 7 days of storage, reaching minimum values both in putrescine-treated and in Ca-treated fruits (near 7 nmol g^{-1} of fresh weight), these ABA levels being significantly lower than those found in control fruits (9.4 ± 0.2 nmol g^{-1} fresh weight). From this time a significant increase was found in the ABA levels in control fruits, reaching a maximum 14 days after storage (25.0 ± 1.4 nmol g^{-1} of fresh weight) and then dropping to half of the concentration. Putrescine-treated and Ca-treated fruits showed also a significant increase of the ABA levels, reaching maximum levels 1 week later than the control fruits.

DISCUSSION

The postharvest storage of fruits includes a number of changes occurring concurrently. This process is accompanied by the softening of the fruit due to a breakdown of the cell wall. We have chosen 15 °C as the temperature of storage because no chilling symptoms could be expected and the fruit metabolism would remain. Our firmness results showed that both putrescine and Ca treatments led to firmer fruits, especially for stage 1 lemons, the immature ones. The use of exogenous polyamines and Ca during the postharvest of fruits to maintain the texture has been reported in apple (Wang et al., 1993; Conway et al., 1994) and strawberry (Main et al., 1986; Ponappa et al., 1993). The effect of increased fruit firmness can be attributed to their cross-link to pectic substances in the cell wall, resulting in rigidification that is detectable immediately after treatment (Abbot et al., 1989). This binding blocks also the access of degradative enzymes to the cell wall, reducing the rate of softening during storage (Conway and Sams, 1987). Polyamine can inhibit also the activity of endopolygalacturonase through binding to pectic acid (Kramer et al., 1989). In Golden Delicious apples Wang et al. (1993) showed that prestorage infiltration with Ca was more effective than that with putrescine in retarding texture changes during storage at 0 °C because the ethylene production was inhibited in this climacteric fruit. However, in the nonclimacteric lemon fruit the effect of the exogenous putrescine was significantly higher than that of Ca during storage at 15 °C (Figure 1a). Also, putrescine-treated fruits led to a significantly lower weight loss during the storage of stage 1 fruits, while Ca-treated and control fruits evolved in a similar way (Figure 2c). During the storage of stage 2 lemons, no differences were found between treated and control fruits (Figure 2d).

Lemon fruits at color break (stage 1) decreased the endogenous putrescine levels throughout the storage period in putrescine-treated fruits and remained higher for Ca-treated fruits. The relative effectiveness of polyamines in retarding senescence and in maintaining the cell wall integrity is thought to be related to the number of positive charges bound to the negative ones of the pectins (Galston and Kaur-Sawhney, 1987). In accordance with this hypothesis, the putrescine exogenously applied went to cell walls to maintain high levels of fruit firmness. We did not quantify this bound

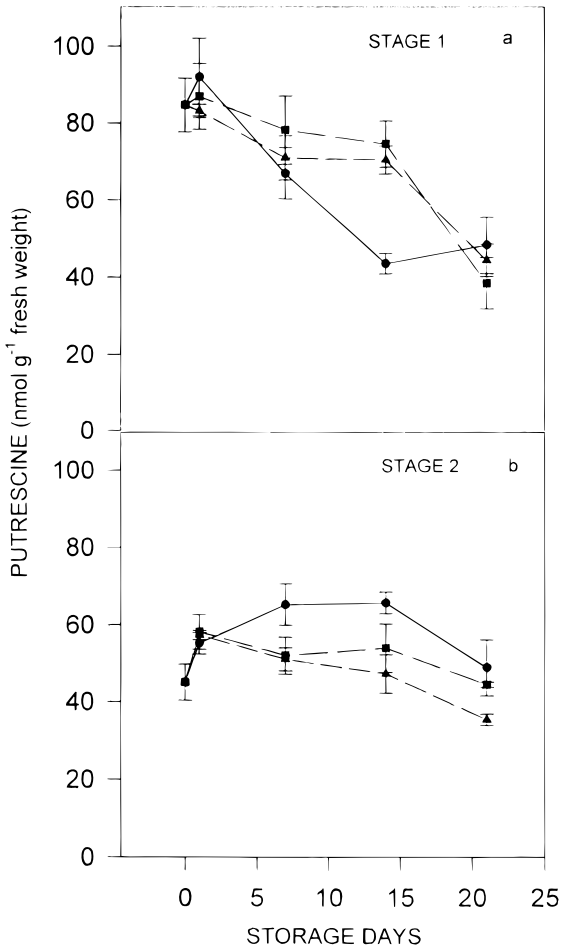

Figure 3. Putrescine changes (nmol g^{-1} of fresh weight) in stage 1 (a) and stage 2 (b) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of determinations made in five fruits in duplicate ± SE.

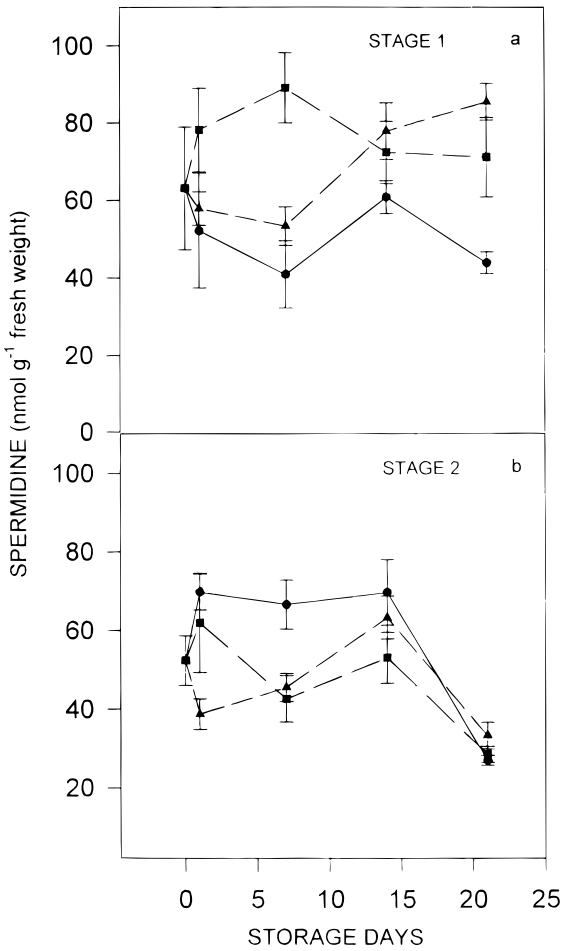

Figure 4. Spermidine changes (nmol g^{-1} of fresh weight) in stage 1 (a) and stage 2 (b) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of determinations made in five fruits in duplicate ± SE.

putrescine, and this could be the reason for the low levels of endogenous free putrescine found after 14 days of storage (Figure 3a). Also, Kramer et al. (1991) have shown in apples that polyamines increased the endogenous levels of polyamines infiltrated, but the levels declined rapidly with storage. On the other hand, the higher levels of free putrescine found in the Ca-treated fruits between the 7th and 14th days of storage might be due to the fact that Ca and polyamines may exhibit a competition for the same binding sites in the cell wall to improve the fruit quality during storage (Wang et al., 1993). In our system the increased fruit firmness after infiltration with Ca is due to the role of this cation in retarding softening in fruits, as has been found in other fruits (Ponappa et al., 1993; Conway et al., 1994), and not increasing the putrescine concentrations.

In stage 2 lemons the same effect of increased fruit firmness after infiltration with putrescine and Ca was observed, but no significant differences were found in relation to control fruits. This can be attributed to the advanced ripening stage, in which degradative cell wall processes have started. In these fruits, high levels of free putrescine in the putrescine-treated lemons were detected, which could indicate a low binding level of the putrescine to the cell wall, and more free putrescine was observed when the fruit reached this ripening stage, that is, a uniform yellow color. In stage 2, only fruits that were infiltrated with putrescine showed an active metabolism from putrescine to spermidine in the biosynthesis pathway, since the levels of this polyamine were higher (Figure 4b), but it could not be detected for spermine levels (Figure 5b). Similar results have been reported in pear, in which spermidine was the polyamine present in the highest concentration and spermine in the lowest, during early storage (Toumadje and Richardson, 1988). However, our results did not show great differences in the spermidine evolution during storage, which could be attributed to the absence of chilling symptoms. This is in agreement with our work on peaches, in which the fruit wooliness was associated with an increase in the spermidine levels (Valero et al., 1997).

Results from ABA evolution showed that both putrescine-treated and Ca-treated lemons had significantly less amount of this plant hormone during the storage as compared with control fruits (Figure 6). These lower ABA levels were accompanied by a delay in the color change in stage 1 lemons, as is shown in Figure 2a. Color development in *Citrus* fruit peel involves temporal changes in chloroplast ultrastructure and the metabolism of both chlorophylls and carotenoids (Richardson and Cowan, 1995). There is well-documented evidence that ABA plays an important role in color development in fruits. Several authors have shown that increased ABA levels in mandarins (Lafuente et al., 1997), oranges (Harris and Dugger, 1986), pepper (Lurie and Ben-

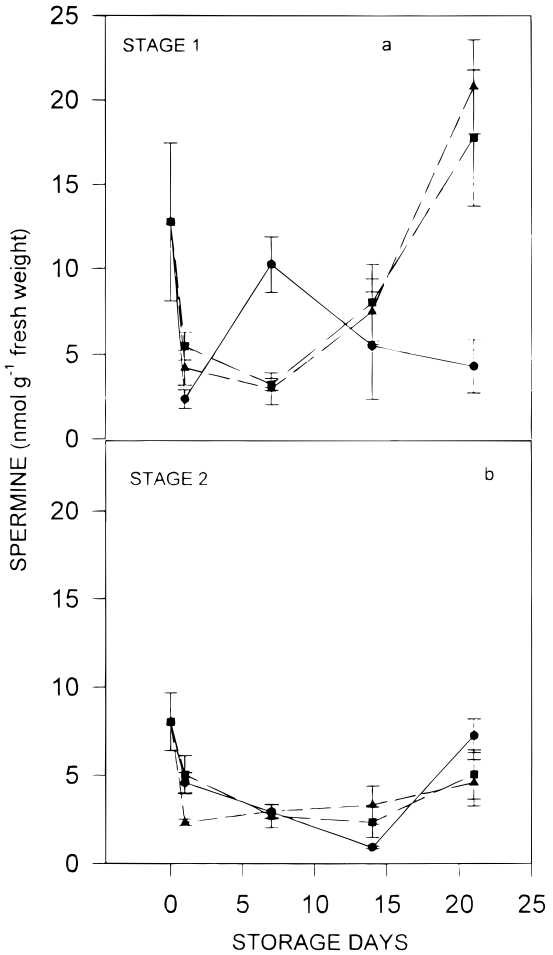

Figure 5. Spermine changes (nmol g^{-1} of fresh weight) in stage 1 (a) and stage 2 (b) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of determinations made in five fruits in duplicate ± SE.

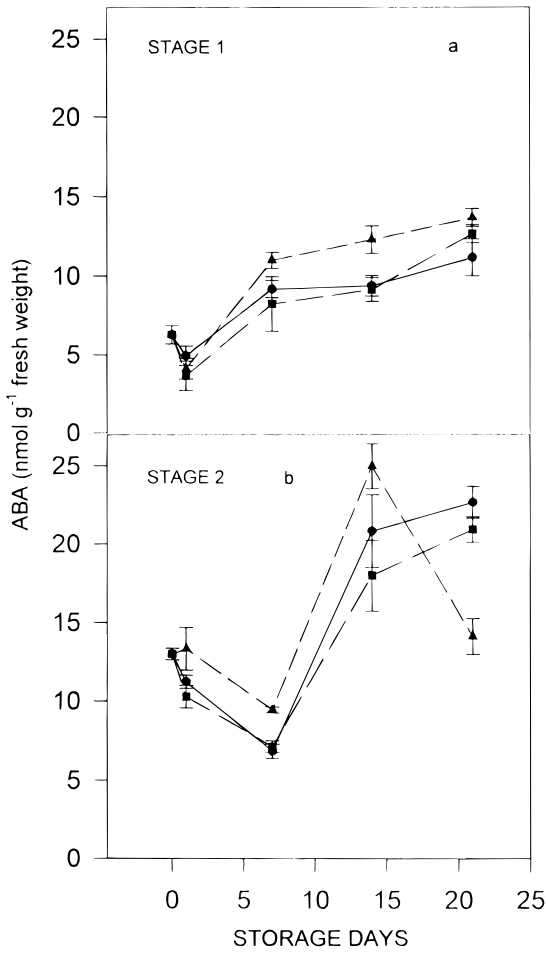

Figure 6. ABA changes (nmol g^{-1} of fresh weight) in stage 1 (a) and stage 2 (b) lemons during storage at 15 °C: 1 mM putrescine (●); 1 mM calcium chloride (■); control (▲). Results represent the mean of quantification made in five fruits in duplicate from three extracts for each fruit ± SE.

Yehoshua, 1986; Serrano et al., 1995), and sweet cherry (Kondo and Gemma, 1993) were responsible for the transition of chloroplast to chromoplast in color development. In stage 2 lemons the same behavior has been found after the infiltration of putrescine and Ca, that is, low ABA levels as compared with control fruits (Figure 6b). However, this ABA is not related with color development, because these lemons were harvested in the ripe stage and the color did not change during storage (Figure 2b). The peak of ABA found in control fruits after 14 days of storage could be related to the beginning of the senescence processes, since ABA declines during late senescence (Nooden, 1988). The low ABA levels found in both putrescine-treated and Ca-treated lemons might be correlated with the physiological role of these naturally occuring compuonds in retarding senescence (Galston and Kaur-Sawhney, 1987; Poovaiah, 1988).

In conclusion, vacuum infiltration with putrescine was more effective than that with calcium to retard the senescence process of the lemon fruit at the early ripening stage, which induced increased fruit firmness and lowered weight loss. Both treatments delayed the color change, which could be related to the lower levels of ABA during storage. However, the role of polyamines and calcium in the overall balance within the postharvest physiology of lemons deserves further study, such as the changes in conjugated polyamine content and the polyamine bound to the cell wall.

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