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# Calcium Delays the Postharvest Ripening and Related Membrane-Lipid Changes of Tomato

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## Abstract

Effects of foliar $CaCl_2$ treatment on postharvest tomato (*Lycopersicon esculentum* Mill.) ripening and senescence as well as on membrane lipid degradation were assessed during two separately carried out experiments. In the first one, foliar $CaCl_2$ application on two cultivars Trésor and H 63-5, caused a significant increase in fruit Ca content and consequently slows the diminution of firmness and acidic citric content, the increase of pH, and the development of red color and gray mold during storage. In addition, $CaCl_2$ application had more effect on the softer H 63-5 fruit, which contained relatively low level of Ca at the time of treatment. In the second experiment calcium implication in cellular membrane stabilization of cultivar Caruso has been verified. Chlorophylls content decreased and that of the carotenoids increased during ripening and senescence, in correlation with of phospholipids reduction. In conclusion, the Ca delays tomato ripening and senescence during storage. This could be probably by protection of membrane lipids from degradation.

**Keywords:** Tomato fruits; $CaCl_2$; Ripening; Senescence; Storage; Lipid degradation

## Introduction

The involvement of Ca in the regulation of fruit maturation and ripening is well established [1,2]. It has been reported that fruit containing low Ca level are sensitive to many physiological and pathological disorders, and consequently have short shelf-life [3-6]. Thus, Ca applying before/after harvest prevents physiological disorders, increase resistance to diseases, delays ripening and subsequently, improves quality of fruit crops [6-9]. Ca treatment has been shown to decrease respiration, reduce ethylene production and to delay the onset of ripening of apple, strawberry, avocado and mango [1,6,10-13]. Preharvest calcium sprays may slightly increase fruit calcium content and this increase may differ from year to year, depending on actual environmental factors [8]. Nevertheless, the success of attempts to increase Ca levels in some fruit by preharvest $CaCl_2$ spray has been limited [12,14].

Studies highlighting the mechanism of action of Ca showed that the Ca may affect the structure and function of cell walls and membranes and certain aspects of the cell catabolism [15-18]. Indeed, the loss of cell membrane integrity is characteristic of plant senescence [19]. This is evident from progressive ultrastructural deterioration and from increased leakage of solutes. Reduced membrane Phospholipids (PL) content during senescence is an index of membrane breakdown as shown for senescing cabbage leaves [17]. This effect can be delayed by Ca, either by preharvest or postharvest application [4]. However, some studies report acceleration of senescence [1,17]. The excessive increase in cytosolic Ca level could be stimulate lipolytic enzyme and accelerate deterioration of membranes [17,19]. Although, Ca effects on fruit senescence has been demonstrated, only few studies show the effectiveness of pre-harvest $CaCl_2$ treatment on the preservation of quality and the prevention of physiological disorders during the storage of tomato (*Lycopersicon esculentum* Mill.) at low temperatures. Indeed, after harvest, the fruit ripens quickly. This may be responsible for fruit short life and represents a serious constraint for efficient handling and transportation. Therefore, losses are often significant [20].

The present study compare the effects of foliar application of CaCl2 on fruit Ca content, fruit ripening and susceptibility to mold development of two tomato cultivars differing in firmness. In addition, this study investigates whether the delay of tomato ripening, caused by Ca treatment, was related to lipid membrane protection from degradation.

## Materials and Methods

Two experiments were carried out to evaluate the $CaCl_2$ effects on tomato (*Lycopersicon esculentum* Mill.) preservation and cell membrane degradation. In the first experiment, where the Ca effects on tomato, cvs. Trésor and H 63-5, maturation and senescence was verified, plants were treated with $CaCl_2$ by foliar application, 16, 8 and 3 days before harvest at dose 0 or 10 kg/ha. They were fertilised according to the recommendations of the Ministry for the Agriculture of Tunisia. Plants were grown on a sandy-loam soil at a spacing of 60 cm between plants, 1 m between row, and 2 m between plots. Immediately after harvest, fruit was pre-cooled and selected for uniformity of size and color (one-forth to one-half red) and lack of wounding. Tomatoes were stored in 26-liter polyethylene containers under a continuous air flow at 12°C and close to 100% RH during 21 days in dark. They were 9 lots of 16 tomatoes in each container. The composition of the container atmosphere was checked by gas chromatography (model 29, Fisher-Hamilton Gas practitioner; Ottawa, Ont.). Ca was determined in fruit by atomic spectrophotometry (Perkin Elner, Analyst 300, USA). The fruit samples were dried at 70°C and digested with nitric and perchloric acids [21]. Ripening after harvest was assessed by measurement of free sugar, titratable acidity, pH, color, firmness and by visual rating of

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mold development. For determination of free sugar, titratable acidity and pH, eight fruit homogenates per replication were filtered through layers of cheese cloth to obtain clear juice. Free sugar content was recorded by a refractometer (Bausch and Lomb optical series YB 3301; Bausch and Lomb, Rochester, N.Y.). Results were expressed as percent free sugars. Titratable acidity was determined as described by Morris et al. [22] by titrating the fruit juice, after diluting with distilled water, against 0.1N NaOH solution using phenolphthalein as an indicator to the end point at pH 8.1. Results were expressed in terms of percentage citric acid [23]. The pH was evaluated by pH-meter (Metrohom 744) according to the method described by Chéour et al. [24]. Electrodes were directly immersed in a juice. Fruit firmness was determined on 8 tomatoes per replicate, as described by Lana et al. [25] with Universal Testing Machine (Instron, model 4411, Canton, Mass., USA). Firmness was measured via compression using 0.05 kN load cell and stainless steel, 7.5 mm diameter convex probe. After establishing zero-force contact between the probe and the horizontally positioned fruit, specimens were compressed 2.5 mm at the equatorial region of each fruit. The maximum force (N) generated during the probe travel used for data analysis. Measurements were made at two equidistant points on the equatorial axis of each of 12 fruits. Color was determined on 8 fruits as tomato color index (TCI) according to the formulate a/L x [L/(a2 + b2)], of Hunter L, a, b method (Colorgard 1000; Pacific Scientific, Silver Spring, Md.). The hunter "*a*" value ranges from green (negative) to red (positive). The hunter "*b*" value ranges from blue (negative) to yellow (positive) [26]. Mold was estimated visually, using a scale from 0 to 9. 9 indicating completely mold covered fruits. Results are means of 16 tomatoes per replicate. All measurements were performed at 20°C.

In the second experiment, where the implication of Ca in the stabilization of the cellular membranes was verified, Phospholipids (PL), Free Sterols (FS) and Free Fatty-Acids (FFA) were determined during ripening of the cultivar Caruso. Plants were grown under the same conditions of the experiment 1. The layout of the plots was also similar. Plants were treated with 0 or 10 kg $CaCl_2$/ha at 9, 6 and 3 days before harvest. The storage has been made according to conditions described in the first experiment. The ripening of the tomatoes was evaluated by determination of chlorophylls (Chl) and carotenoids contents as described by Bergevin et al. [27]. Extraction was done by a chloroform and methanol mixture (2/1, v/v) and the absorbance was measured at 480 and 664 nm by a spectrophotometer (Halwett Packard, Model 8451A Diory Arry). Pure Chl B and lycopene solutions were used to plot the standard curve. Pericarps were fixed in boiling water for 3 min to inactivate endogenous phospholipases.

Total lipids were extracted from tissue conforming to Blight and Dayer procedure [28]. The lipids in the chloroform phase were separated by TLC on 250 µm silica gel G plates (Fisher Scientific Co., Ottawa, ON). Acetone/acetic acid/water (100/2/1, v/v) was used to separate the PL from galactolipids, hexana/diethyl ether/acetic acid (80/20/1; v/v/v) was used to separate the neutral lipids, and chloroform:methanol/acetic acid/water (80/15/15/3.5, v/v/v/v) was used to separate PL. The lipids were visualized in iodine vapors and identified using authentic standards (Sigma, St-Louis, MO). The area corresponding to each class on the TLC plate was scraped into a test tube and transmethylated directly onto the silica gel with 14% (w/v) BF3 in methanol [29]. For quantitative determination of FA, a known amount of heptadecanoate (C17:0) was added as an internal standard. Methyl esters of FA were analyzed by GLC (Hewlett-Packard, model 5890A, Mississauga, ON) on a 30-m capillary DB 225 column (J & W Scientific, Rancho Cordova, CA) as described by Makhlouf et al. [30]. FS were silylated directly on the silica gel [31] and assayed by GLC using cholestane as a standard. Sterol trisilyl derivatives were separated by GLC on a 25-m capillary column (Hewlett-Packard, ULTRA 1, Mississauga, ON). Lipoxygenase (LOX) activity was determined spectrophotometrically at 234 nm [32]. The standard assay mixture contained 1.5 mM linoleic acid and 0.5% (v/v) Tween 20 in 30 mL Pipes buffer (pH 7). A 0.5-mL aliquot of the extract was added to 2 mL of reagent in a cuvette.

Analysis Of Variance (ANOVA) of results was made following a factorial randomly complete block design [33] by the GLM procedure of the SAS statistical package [34]. The sources of variation were cultivar, $CaCl_2$ rate and time of storage, and their interactions for the first experiment. Homogeneity of variance was verified by the standard Bartlett test [35]. Each treatment was randomised on three blocks. The two experiments were repeated twice and only the results of the seconds are presented.

## Results

### Effect of Ca on tomatoes maturation

Foliar application of $CaCl_2$ caused an increase in Ca level of fruits in both cultivars (Table 1). However, the Ca content increased more in H 63-5, which had shown before $CaCl_2$ application a relatively lower Ca level, than in Trésor ($P<0.05$). Free sugar increased during storage for both tomato cultivars. This increase was not delayed by Ca for both cultivars ($P > 0.05$) (results not shown). Organic acids, expressed as citric acid, increased at the beginning of storage and decreased at the end in both cultivars (Figure 1). Ca treatment delayed this change but more so with H 63-5 ($P<0.05$). The pH increased for two cultivars during storage (Figure 2). The increase was delayed by $CaCl_2$ treatment for both cultivars but the effect was more marked for H 63-5 ($P<0.05$). The pH of tomatoes treated with $CaCl_2$ was more acid at harvest and during storage. Color, expressed in TCI, increased gradually for both cultivars to about the same level during storage (Figure 3). The effect of Ca was observed as from the 7th day of storage for both cultivars but it was more pronounced in the case of H 63-5 ($P<0.01$). With time of storage less force was required to compress the fruit (Figure 4). The decrease in firmness was delayed by calcium treatment for both cultivars. The effect of calcium treatment was more observed at harvest and throughout the storage period for H 63-5 ($P<0.05$). Mold developed more quickly on H 63-5 than on Trésor (Figure 5). Ca treatment caused a delay in mold development but the effect was clearer on H 63-5 ($P<0.01$). For all these maturity characteristics, the 'cultivar x Ca x storage' interaction was not significant which indicated that the cultivars responded in the same way to $CaCl_2$ treatment even if H 63-5 reaction were slightly more important ($P > 0.05$).

### Effects of Ca on the membrane lipids degradation

Chl content of pericarp decreased significantly during storage of fruits at 12°C (Table 2). This decrease was accompanied by a significant increase in carotenoids content. However, $CaCl_2$ treatment delayed these changes. PL and FS contents were measured during storage to

| Cultivars | $CaCl_2$ (kg/ha) | Ca content (mg/100g FW) |
|---|---|---|
| Trésor | 0 | 10.4 ± 0.3 |
| | 10 | 11.0 ± 0.8 |
| H 63-5 | 0 | 08.9 ± 0.6 |
| | 10 | 11.1 ± 0.5 |

$CaCl_2$: Dichloride Calcium; FW: Fresh Weight.

**Table 1:** Calcium content (%FW) of tomato pericarps, cvs Trésor and H 63-5, after foliar application of $CaCl_2$. Values are means ± SD for 3 replicates.

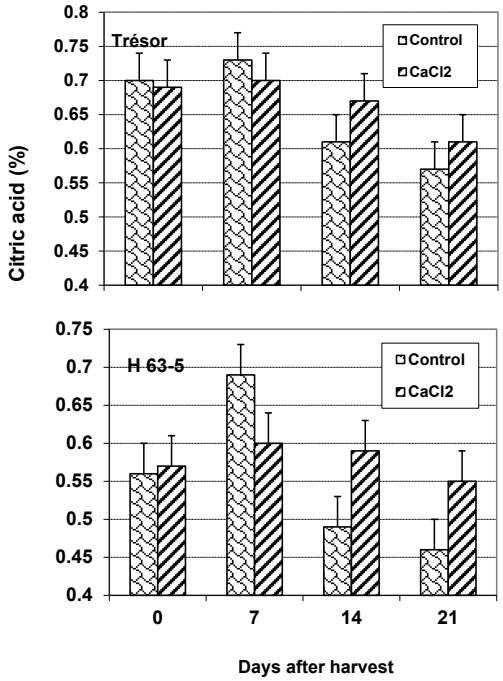


**Figure 1:** Titratable acidity (citric acid, %FW) of 'Trésor' and 'H 65-3' tomatoes stored in darkness under a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. Vertical lines show average SD for 3 replicates.

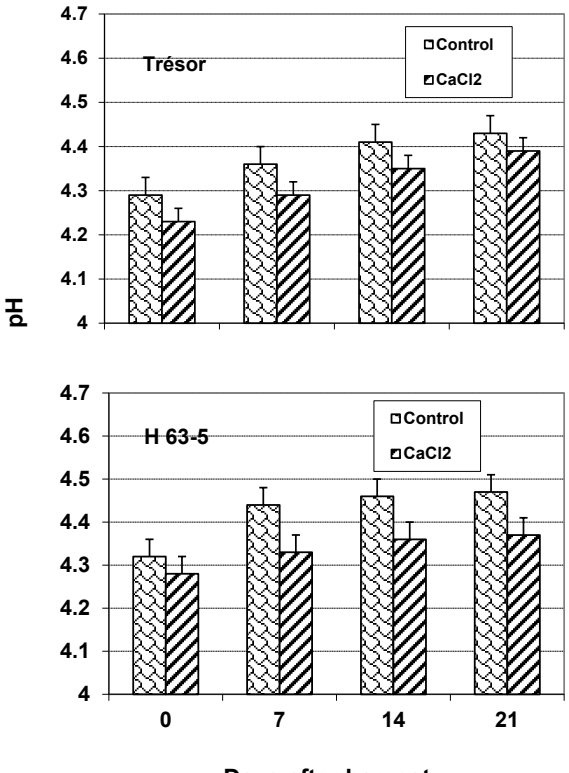


**Figure 2:** pH of 'Trésor' and 'H 65-3' tomatoes stored in darkness under a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. Vertical lines show average SD for 3 replicates.

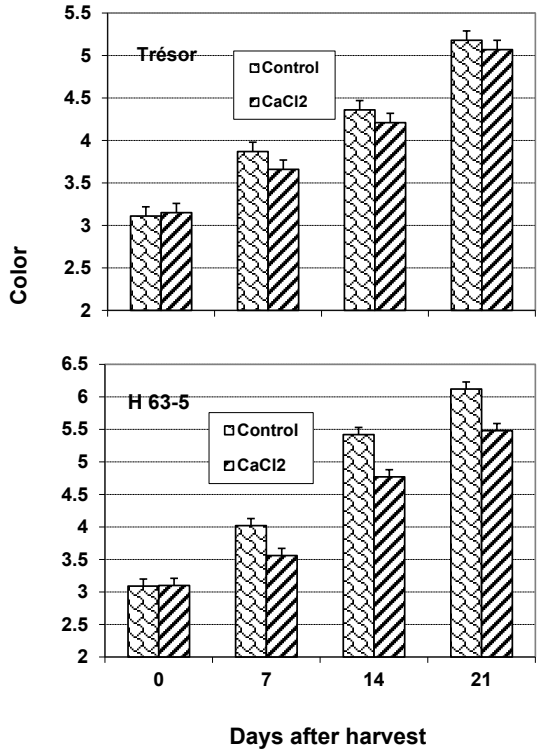


**Figure 3:** Color of 'Trésor' and 'H 65-3' tomatoes stored in darkness under a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. Vertical lines show average SD for 3 replicates.

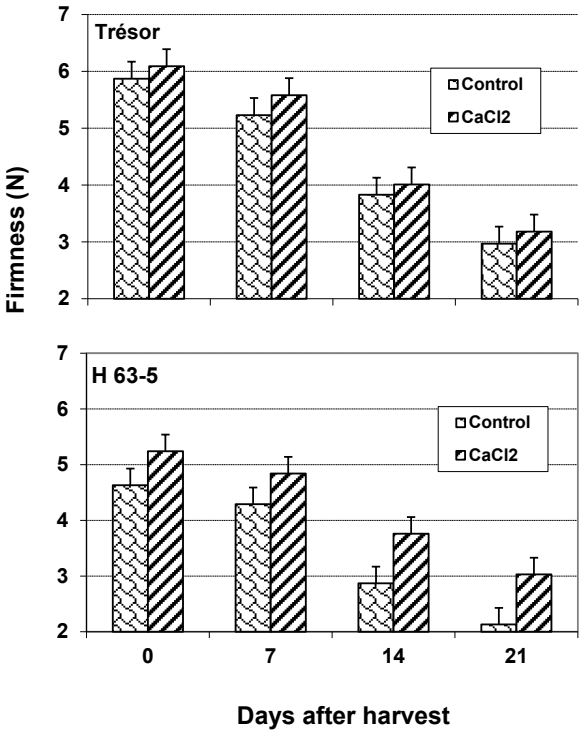


**Figure 4:** Firmness of 'Trésor' and 'H 65-3' tomatoes stored in darkness under a continuous air stream at 12°C and close to 100% RH for 21 days after foliar CaCl2 application. Vertical lines show average SD for 3 replicates.

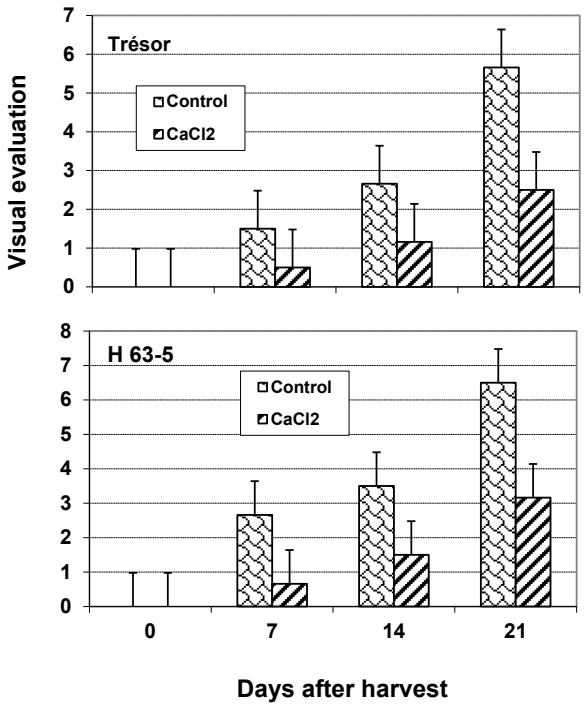


**Figure 5:** Visual assessment of mold development on 'Trésor' and 'H 65-3' tomatoes stored in darkness under a continuous air stream at 12°C and close to 100% RH for 21 days after foliar CaCl2 application. Vertical lines show average SD for 3 replicates.

| Treatment | Days | Chlorophylls (nm/g FW) | Carotenoids (nm/g FW) | PL (nm/g FW) | FS/PL (µg / µg) |
|---|---|---|---|---|---|
| Control | 0 | 10.6 ± 0.7 | 7.2 ± 0.09 | 179.2 ± 26.0 | 0.15 ± 0.01 |
| | 14 | 0.3 ± 0.1 | 92.0 ± 21.6 | 156.1 ± 33.2 | 0.26 ± 0.04 |
| | 21 | ≤ 0.1 | 167.2 ± 37.9 | 141.6 ± 36.6 | 0.37 ± 0.03 |
| $CaCl_2$ | 21 | ≤ 0.1 | 167.2 ± 37.9 | 141.6 ± 36.6 | 0.37 ± 0.03 |
| | 21 | 0.9 ± 0.2 | 102.7 ± 21.3 | 166.1 ± 33.9 | 0.21 ± 0.02 |

$CaCl_2$: Dichloride Calcium; FS/PL: Free Sterol/Phospholipids; FW: Fresh Weight; RH: Relative Humidity; PL: Phospholipids.

**Table 2:** Change with time in Chlorophylls, Carotenoids and PL contents, and FS/PL ratio of pericarp tissue during storage in darkness of tomato fruit, cv. Caruso, in a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. Values are expressed as means ± SD.

determine whether the changes in Chl and carotenoids contents were associated with an alteration in membrane lipid composition. Total PL content declined during storage for all treatments (Table 2). The rate of decline in PL level was less important with fruit treated with Ca. Water fruit content does not change during storage. The most important PLs were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositole and the phosphatidylglycerole (48, 37, 8 and 3%, respectively). The proportion of PL classes did not change significantly during storage which indicated that each class of PL declined at a similar rate ($P > 0.05$). The total content of sterols on a fresh weight basis showed no significant change under any treatment during ripening. The loss of membrane PL from was reflected by a shift in the FS to PL ratio (Table 2) ($P<0.001$). The FS/PL ratio, which increased significantly for both treatments but hardly for control, was closely correlated to the loss of Chl and increase of carotenoids contents (r=-0.64 and 0.94, respectively). Tables 3 and 4 showed the composition of the PL and FFA fractions. The PLs were rich in linolenic acid, and their ratio of PUFA to saturated FA (mol%), 2.69, was greater than that of the FFA, 2.53. Loss of PUFA from both fractions during ripening was reflected by a decrease in the ratio of PUFA to saturated FA. The decrease was greater for control than in the fruit treated with Ca ($P<0.001$).

The loss of PUFA from FFA and their low level in FFA fraction suggested LOX involvement in lipid breakdown in tomato. When analyzed, LOX specific activity has been indeed steadily increased in the control (Figure 6) ($P<0.001$). The increase was less for the fruit treated with Ca.

## Discussion

The beneficial effect of Ca on the delay of ripening and senescence of some fruit and vegetables has been demonstrated [1,2]. Indeed, the loss of firmness and the preservation of quality during storage of apple and pear were often associated with a Ca deficiency. The paucity in this element is also associated with several physiological disorders such as the bitter pit in apple and the apical rot in tomato [14,36]. However, to generalize its application for other horticultural products, specific studies are necessary. In fact, the fruit and vegetables are characterized by their diversity and consequently their reactions to Ca could be different [6].

Characteristic symptoms of ripening, increase in free sugar, pH and mold development; and decrease in titratable acidity and firmness, were observed during storage of Trésor and H63-5 cultivars tomatoes at 12°C. Foliar application of $CaCl_2$ a few days before harvest caused an increase in a Ca content of the tissues and consequently influenced some of these parameters, and delayed ripening and prolonged storage life of tomatoes as demonstrated by Wills and Tirmazi [37]. Such observations were reported on strawberry, apple and pear [6,38,39].

| Treatment | Days | FA | | | | PUFA/S |
|---|---|---|---|---|---|---|
| | | 16:0 | 18:1 | 18:2 | 18:3 | |
| Control | 0 | 25.2 ± 0.8 | 1.9 ± 1.2 | 52.7 ± 1.8 | 13.2 ± 1.5 | 2.69 ± 0.03 |
| | 14 | 27.9 ± 1.2 | 5.3 ± 1.3 | 51.2 ± 1.3 | 9.6 ± 1.2 | 2.37 ± 0.02 |
| | 21 | 29.2 ± 1.0 | 7.2 ± 0.6 | 50.3 ± 1.4 | 7.9 ± 1.6 | 2.21 ± 0.02 |
| $CaCl_2$ | 14 | 26.3 ± 1.3 | 3.1 ± 0.3 | 51.9 ± 1.6 | 12.7 ± 1.1 | 2.57 ± 0.03 |
| | 21 | 26.8 ± 0.9 | 4.9 ± 0.3 | 51.1 ± 1.2 | 9.4 ± 1.0 | 2.44 ± 0.02 |

$CaCl_2$: Dichloride Calcium; FA: Fatty Acid; PUFA/S: Polyunsaturated Fatty Acid/Sterol; RH: Relative Humidity

**Table 3:** FA composition (mol%) of the PL fraction of pericarp tissue during storage in darkness of tomato fruit, cv. Caruso, in a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. Values are expressed as means ± SD.

| Treatment | Days | FA | | | | | PUFA/S |
|---|---|---|---|---|---|---|---|
| | | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 | |
| Control | 0 | 25.9 ± 0.8 | 1.9 ± 1.2 | 3.7 ± 0.8 | 46.9 ± 1.5 | 19.6 ± 1.6 | 2.53 ± 0.03 |
| | 14 | 28.2 ± 1.2 | 2.6 ± 0.9 | 7.4 ± 1.3 | 50.6 ± 1.2 | 8.7 ± 0.9 | 2.17 ± 0.02 |
| | 21 | 30.1 ± 1.0 | 3.7 ± 0.6 | 10.9 ± 1.4 | 53.3 ± 1.6 | 6.2 ± 1.1 | 2.02 ± 0.03 |
| $CaCl_2$ | 14 | 26.1 ± 1.3 | – | 4.2 ± 1.3 | 47.7 ± 1.1 | 14.1 ± 1.6 | 2.53 ± 0.02 |
| | 21 | 27.9 ± 0.9 | 2.7 ± 0.3 | 6.9 ± 1.2 | 48.4 ± 1.0 | 11.7 ± 0.9 | 2.26 ± 0.02 |

$CaCl_2$: Dichloride Calcium; FA: Fatty Acid; FFA: Free Fatty Acid; PUFA/S: Polyunsaturated Fatty Acid/Sterol.

**Table 4:** FA composition (mol%) of the FFA fraction of pericarp tissue during storage in darkness of tomato fruit, cv. Caruso, in a continuous air stream at 12°C and close to 100% RH for 21 days after foliar $CaCl_2$ application. PUFA/S. Values are expressed as means ± SD.

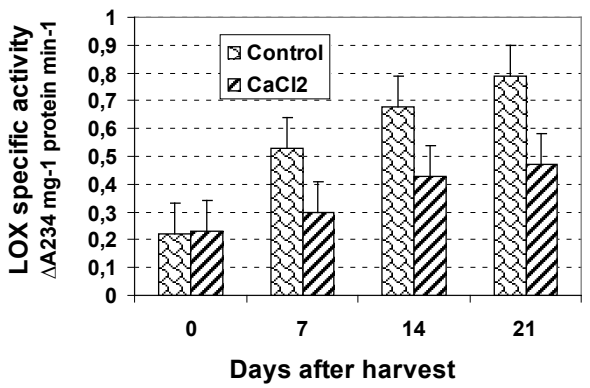


**Figure 6:** Change with time of LOX activity of pericarp tissue during storage in darkness of tomato fruit, cv. Caruso, in a continuous air stream at 12°C and close to 100% RH for 21 days after foliar CaCl2 application. Vertical lines show average SD for 3 replicates.

However, the response of the Ca treatment was greater for H 63-5, which contained less Ca than Trésor. The effect of Ca is less pronounced in tissues that contain adequate amounts of Ca for maintaining cell integrity [40]. The ability to accumulate and distribute Ca may vary with the cultivar and is FFinfluenced by various factors such cultivar, temperature, relative humidity, plant age, levels of other minerals in the soil (Ferguson 1984) (1). The inability of plants to accumulate and distribute Ca may partly explain why some are more prone to disorders and diseases [41].

A characteristic feature of senescence is membrane deterioration due to lipid degradation and the ensuing destabilization of the bilayer. The protection of cell membrane integrity by Ca during senescence has been explained by the ability to binding to membrane phospholipids and, in this way, to stabilize the membrane and to control membrane-associated functions [19]. Our results showed that tomato ripening, reflected by loss of Chl and increase in carotenoids, was delayed by Ca application as reported previously by Chéour et al. [17] for cabbage leaves. Lipid membrane breakdown in tomato during senescence was indicated by several markers: reduced PL content, larger ratio FS/PL and reduced rates of PUFA of the fractions PL and FFA. The levels of these markers changed in parallel with Chl degradation and carotenoids increase which are common indicators of tomato senescence. Membrane Protection from lipid degradation by Ca was explained by the decrease in most of these changes. The present results, based on the levels of intermediate products of PL breakdown, should be interpreted in the context of steady-state equilibrium between synthesis and degradation. Chéour et al. [17] have reported that the Ca can stabilize the plasmalemma by binding to the phospholipids. In fact, membrane becomes less prone to degradation by lipolytic enzymes. The undegraded lipid bilayer prevents Ca from passively entering the cytosol and facilitates the pumping of Ca outside of the cytoplasm by membrane-associated $Ca^{2+}$-ATPase. A low Ca level in the cytosol is essential for the normal functioning of cell metabolism. When the cytosolic Ca concentration increases, it interferes with normal biochemical activities by activating or deactivating numerous enzymes, either directly or indirectly, through various mechanisms involving a change in protein conformation, protein phosphorylation, or interaction with calmodulin [4] The constancy of the proportions of the PL classes during ripening shows that the different PLs were degraded at similar rates. The loss of PUFA from the PL classes during storage shows that the polar head-groups may have less influence on PL degradation than the FA composition of the molecular species. Such observation confirms previous reports on cabbage by Chéour et al. [17]. The marked rise in the relatively saturated FFA content, the progressive decrease in degree of unsaturation of the PL and FFA fractions, and the increasing LOX specific activity are ample evidence that LOX was involved in membrane lipid breakdown during tomato senescence. LOX (EC 1.13.11.12) is a dioxygenase that catalyzes the peroxidation of fatty acids containing a cis-cis-1, 4 pentadiene configurations [42]. Our results indicate that Ca treatment influenced its activity during tomato senescence. The decrease in the level of PUFA in the PL and FFA fractions, sign of LOX activity, was delayed by Ca treatment suggesting lower LOX activity, which was confirmed by enzyme assay.

In conclusion, foliar application led to increased Ca content of tomato fruits and delayed ripening and mol development. The response of $CaCl_2$ treatment varied with cultivar and apparently depends on the Ca content of the fruit at the time of treatment. The presence of Ca probably implies a protection of membrane lipids from degradation. Lipoxygenase, responsible for the peroxidation of the polyunsaturated fatty acids, was probably influenced by calcium in the tomato fruit.

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