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A calcium lactate treatment at harvest, growing system and refrigerated modified atmosphere can affect strawberry's ´Camarosa´ postharvest quality?

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Key words: nutritional quality, organoleptic quality, perlite, postharvest behaviour, soil, storage temperature.

Abstract: The aim of this work was to evaluate the effect of a calcium lactate treatment on postharvest behaviour, organoleptic and nutritional quality of strawberries (*Fragaria x ananassa* Duch., cv. Camarosa) grown in different growing systems and stored in refrigerated modified atmosphere. Strawberry grown in perlite and soil in greenhouse and soil in open field was harvested and dipped in a calcium lactate 1% solution. Fruits were packed in modified atmosphere at 1°C and 8°C. At 1, 3 and 7 days of storage postharvest behaviour, organoleptic and nutritional quality was evaluated. Calcium had a positive effect on fruit firmness and no differences were observed between storage temperatures in calcium treated fruits. Organoleptic quality (except visual quality) was better in fruits grown in open field soil, regardless calcium treatment and storage temperature. Nutritional quality was better in untreated fruits and stored at 1°C.

1. Introduction

Strawberry (*Fragaria x annanassa* Duch.) is one of the most important berries produced in the world. In Argentina, 33000 tons are produced, mainly cultivar Camarosa (Gómez Riera *et al.*, 2013). It is highly accepted by consumers firstly because of its physical aspect and organoleptic and nutritional properties (Shin *et al.*, 2008; Garriga *et al.*, 2015). Nevertheless, its postharvest quality rapidly declines due to the soft texture, high metabolic activity and susceptibility to bacterial and fungal rots. Usually, there is quality decay during transport and commercialization (Dotto *et al*., 2011).

Cell wall degradation is one of the principal causes of strawberry´s postharvest quality decay. As pectine synthesis occurs along the fruit maturation, they are less firmly attached to the cell wall. Additionally, middle lamella´s debilitation and solubilization during fruit ripening diminishes cell cohesion (Lara *et al.*, 2004).

Calcium plays a preferential role on permeability and cell integrity and has a direct influence on fruit firmness and storage time (Fernández *et al.*, 2006). Calcium functions as an intracellular cement because it forms calcium-pectine complexes that give firmness to vegetable tissues. Calcium´s presence also favours pectic material insolubilization and inhibits its degradation by polygalacturonase enzyme (Alonso, 1995).

An immersion in calcium at harvest could increase calcium´s content in strawberry, increasing fruit firmness (Galetto *et al.*, 2010) and thereby storage period. However, the key factor for quality maintenance is temperature - optimum for strawberry is 0°C (Mitcham *et al*., 2015). Could an immersion in calcium allow an increase in storage temperature, maintaining fruit quality?

Other factors, such as growing system and substrate, can influence strawberry´s quality. Greenhouse production improves organoleptic quality of fruits and vegetables because they are not exposed directly to air conditions as in open field (Gruda, 2009). Soilless production, moreover, diminishes incidence of diseases and pests (Urresterazu, 2004).

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The aim of this work was to evaluate the effect of a calcium lactate treatment on postharvest behaviour, organoleptic and nutritional quality of strawberries grown in three growing systems and stored at two temperatures in modified atmosphere during 7 days of storage.

2. Materials and Methods

Plant material and growth conditions

The experiment was conducted in the Horticulture experimental field, School of Agriculture, University of Buenos Aires (latitude 45° S, longitude 58° 31´ W, altitude 26 m asl). Strawberry seedlings (commercial variety 'Camarosa') were planted in three growing systems: soil in open field, soil and perlite in greenhouse. Plant density was 7 plants m^{-2}. Soil treatments were covered with a black plastic mulching. All treatments were fertirrigated: nutrient solutions were formulated according to strawberry´s requirements (Table 1). Hourly intervals of temperature (°C), relative humidity (%) and radiation ($W \cdot m^{-2}$) were measured in open field and greenhouse with datalogger HOBO.

Table 1 - Nutrient solution formulation

Macroelements		Microelements	
Element	Concentration (mg l^{-1})	Element	Concentration (mg l^{-1})
Nitrogen	64	Iron	2.8
Phosphorus	31	Sodium	1.2
Potassium	200	Manganese	0.5
Sulfur	64	Boron	0.5
Magnesium	48	Copper	0.02
--		Zinc	0.05
		Molybdenum	0.01

Harvest

Mature strawberry fruits (at least 75% red colour) were harvested and immediately submerged in cold water to decrease fruit temperature. Half of the fruits of each growing system were treated with a calcium lactate 1% solution during one minute. Although calcium chloride is most commonly used, calcium lactate was used as a firming agent (Codex Alimentarius - World Health Organization, 2015) because chloride can give a bitter taste to fruits (Oms-Oliu *et al.*, 2010). Sixty-five grams (g) of fruit were packed in a modified atmosphere (medium density polyethylene semi rigid container). Modified atmosphere increases postharvest life in fruits and vegetables by reducing respiratory rate (Sandhya, 2010). Containers were stored at storage chambers at 1°C and 8°C.

Postharvest

At 1, 3 and 7 days of storage, quality characteristics of fruits were evaluated as follows:

Postharvest behaviour

- Oxygen and carbon dioxide concentration in the container was measured with Dansensor gas analyzer and expressed as percentage.
- Fresh weight loss. Fruits were weighted and weight loss was expressed as percentage relative to the initial value.

Organoleptic quality

- Visual quality. The overall visual and sanitary quality was determined by scoring each strawberry using a 1-10 hedonic scale, being 10 excellent and 6 the commercialization limit.
- Colour was measured with Minolta Chromameter CR300. L, a, b, c, h parameters were determined in four spots in equatorial zone of three fruits per treatment.
- Firmness was measured in equatorial zone with Ludwig penetrometer fitted with a 3 mm diameter round probe.
- Total soluble solids were determined with Atago refractometer and expressed as °Brix.

Nutritional quality

- Ascorbic and dehidroascorbic acid was determined by liquid chromatography and expressed as mg of ascorbic acid 100 g^{-1} of fresh weight.
- Antioxidant capacity. Antioxidant capacity was estimated by determining the free-radical scavenging capacity evaluated with the stable radical DPPH (adapted from Brand Williams *et al.*, 1995 and Leong and Shui, 2002). Two g of edible portion of the fruit was homogenized using a blender and inserted into a 50 ml centrifuge tube. Twenty ml of 50% aqueous ethanol was added (1:10 w/v) and mixed in a vortex mixer for 15-30 seconds. The extract was centrifuged at 2000 g for 5 min al 4°C. The supernatant was filtered before using. A 25 mg solution of DPPH (1,1 diphenyl-2-picrylhydrazyl) was prepared in methanol. For calibration curve, aliquots of ascorbic acid (0, 25, 50, 75, and 100 µl) solved in aqueous ethanol 50% (0.1 ml ml^{-1}) were placed in tubes with in 3 ml DPPH. Absorbance at 517 was measured at 1, 10, 30, 60, 90 and 120 minutes. An aliquot of 50 ml of an antioxidant/fruit extract solution was added to 3 ml of the DPPH solution. The decrease in absorbance at 517

nm was measured at 0, 1, 5 and then every 10 minutes until the reaction reached a *plateau*. The decreased absorbance of DPPH remaining at the steady-state was calculated and expressed as mg of ascorbic acid (AA) equivalents per 100 g of homogenate (AEAC). The AEAC was calculated using the following equation:

$$AEAC=\Delta A \times f \times V \times 100 \times 1/W$$

where Δ A is the change of absorbance after addition of fruit extract, f is the inverse of the calibration curve slope, V is the volume of filtrate (ml) and W is the weight of homogenate used for extraction (g).

Statistical analysis

A completely randomized factorial design with 3 replicates per treatment was used. The results were analyzed by multivariate analysis of variance repeated in time with a 5% significance level. Tukey test to compare means was used (Kuehl, 2001). Infostat software was used (Di Rienzo *et al.*, 2015).

3. Results and Discussion

Oxygen and carbon dioxide concentration in the container

Temperature, as expected, generated the most significant differences: strawberries stored at 1°C respired less than those stored at 8°C (Fig. 1). Nevertheless, in all cases, at equal temperatures, strawberries treated with calcium decreased the respiration.

On the other hand, an interaction between growing systems and calcium treatment was observed ($p<0,0001$) both in oxygen and carbon dioxide levels: strawberries grown in soil systems (both open field and greenhouse) and without calcium treatment showed larger differences between storage temperatures: those stored at 8°C showed a high oxygen decrease and carbon dioxide increase, especially at the 7th day of storage. Treated fruits, instead, showed similar behaviour during the seven days of storage. Thus, calcium treatment could be considered as a regulator because differences between temperatures in treated strawberries were smaller. Waghmare and Annapure (2013) observed that a combination of modified atmosphere, calcium chloride and nitric acid treatment in chopped papayas stored at 5°C had a significant decrease of oxygen and increase of carbon dioxide compared to fruits only stored in modified atmosphere.

Equilibrium modified atmosphere was not established, especially those stored at low temperatures. This could be because medium density polyethylene was used and this material has low permeability to gases: 2600 cm^3/m^2.d.atm for oxygen and 7600 cm^3/m^2.d.atm for carbon dioxide (Sandhya, 2010). Additionally, as temperature increases, material permeability increases as well. Thus, containers stored at 8°C had a higher permeability (Oliveira *et al.*, 2015).

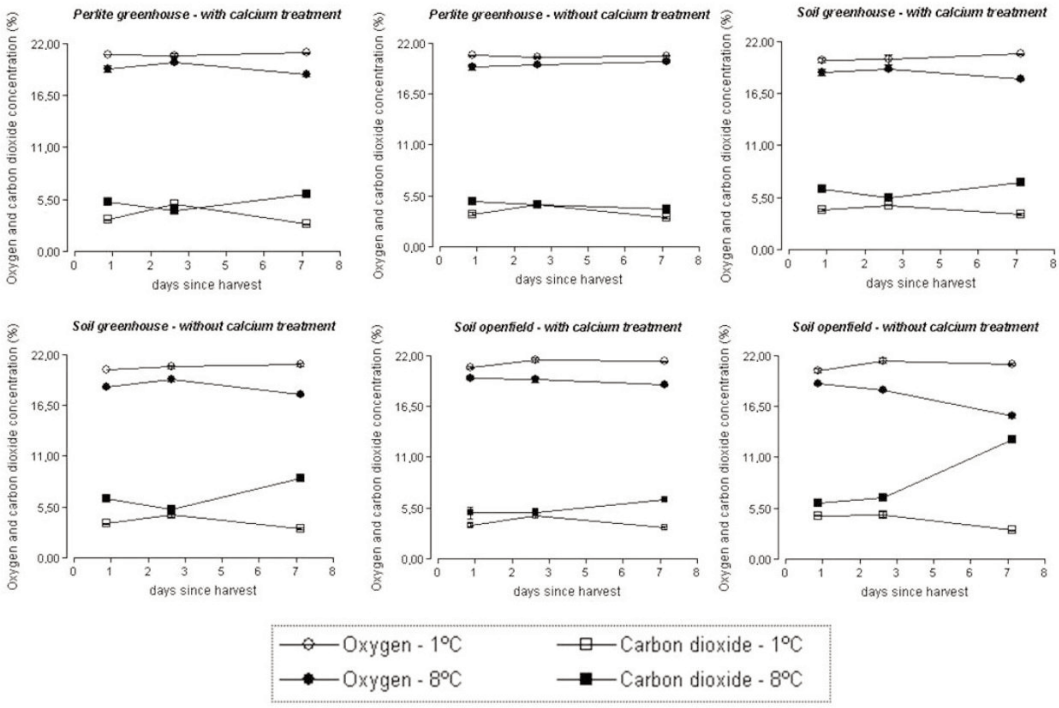

Fig. 1 - Oxygen and carbon dioxide concentration (%) in containers at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures.

Fresh weight loss

Maximum allowable weight loss in strawberry is 6% (Laurin *et al.*, 2003). All treatments, except one case, were below those values (Fig. 2). The smaller weight loss was observed in open field fruits ($p<0.0001$), independently storage temperature and calcium treatment. Preharvest temperatures can affect postharvest shelf life. For example, fruits grown at high temperatures can exhibit water soaking (Benkeblia *et al.*, 2011). In this work, differences between greenhouse and open field temperatures reached 7°C. Fruits harvested in greenhouse soil and soilless systems had surely higher temperature, what determined higher weight loss in those systems.

Visual quality

Fruits grown in perlite had better quality: 7.7% more than open field soil and 20% compared to greenhouse soil fruits (Fig. 3). At the 7th day of postharvest, all the strawberries stored at 1°C pre-

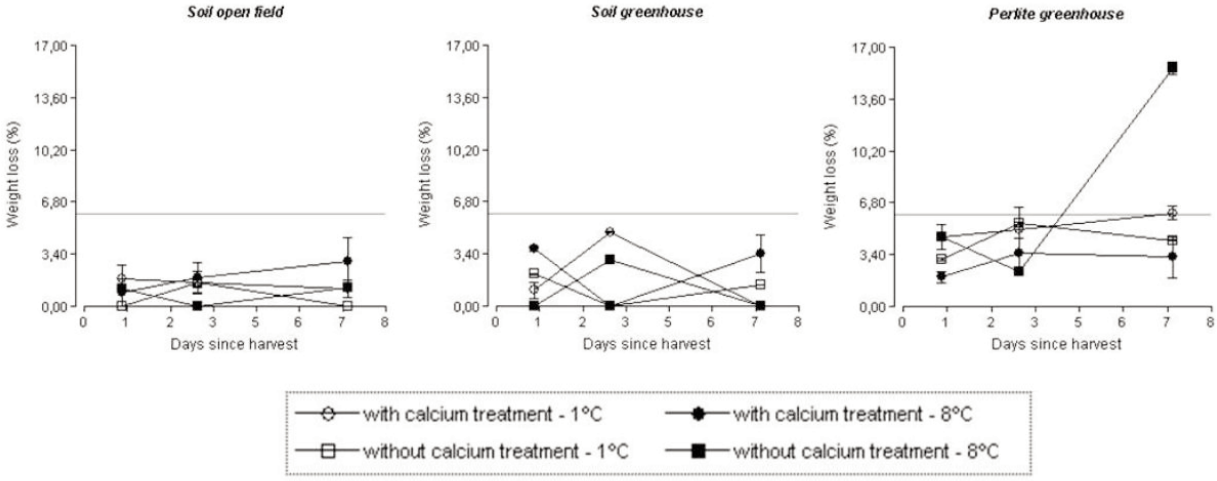

Fig. 2 - Fresh weight loss (%) at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures. Maximum allowable weight loss (6%) is indicated.

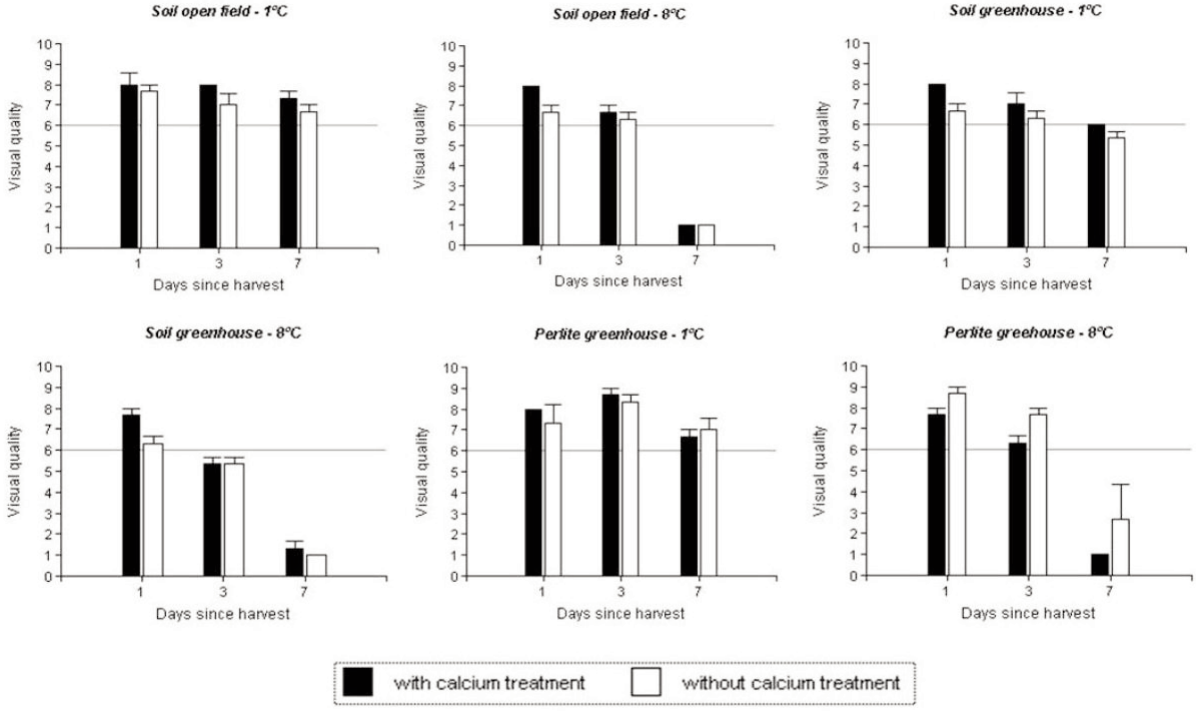

Fig. 3 - Visual quality at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures. Each graph represents a combination of growing system and storage temperature. Commercialization limit (6) is indicated.

sented visual and sanitary quality above commercialization level. Fruits stored at 8°C, instead, were below that level and in most cases they had *Botrytis cinerea* symptoms. Temperature management is a key factor to minimize postharvest deterioration in strawberry. At high storage temperatures, fruits have higher respiration rates, and consequently, shorter postharvest shelf life (Shin *et al.*, 2008).

Colour

Significant differences were not observed for all colour parameters. Shin *et al.* (2008) did not find colour changes during 7 days of storage of L c and h° values.

Firmness

An interaction was observed between growing system and calcium treatment (p=0,0203): treated strawberries grown in soil systems, both open field and greenhouse had 20% more firmness than others. In perlite, difference between treated and untreated fruit was not significant (Fig. 4).

Firmness increased in fruits stored at 1°C along the storage time. Shin *et al.* (2007) also observed an increase in firmness along 4 days of storage at high (10.5°C) and low (0.5°C) temperatures. The same authors investigated firmness during 12 days of storage and observed a positive tendency in fruits stored at 3°C and a decrease in those stored at 10°C (Shin *et al.*, 2008). Firmness increase in low storage temperatures is due to physical changes in cell wall: cold produces an increase in pectin viscosity, which impacts positively in fruit firmness (Lara *et al.*, 2004).

Total soluble solids

Total soluble solid content (Table 2) in all cases was above the minimum content recommended (7°Brix) for the postharvest quality maintenance (Mitcham *et al.*, 2015). A significant interaction was found between temperature and growing system (p<0,0001): fruits stored at 8°C and grown in open

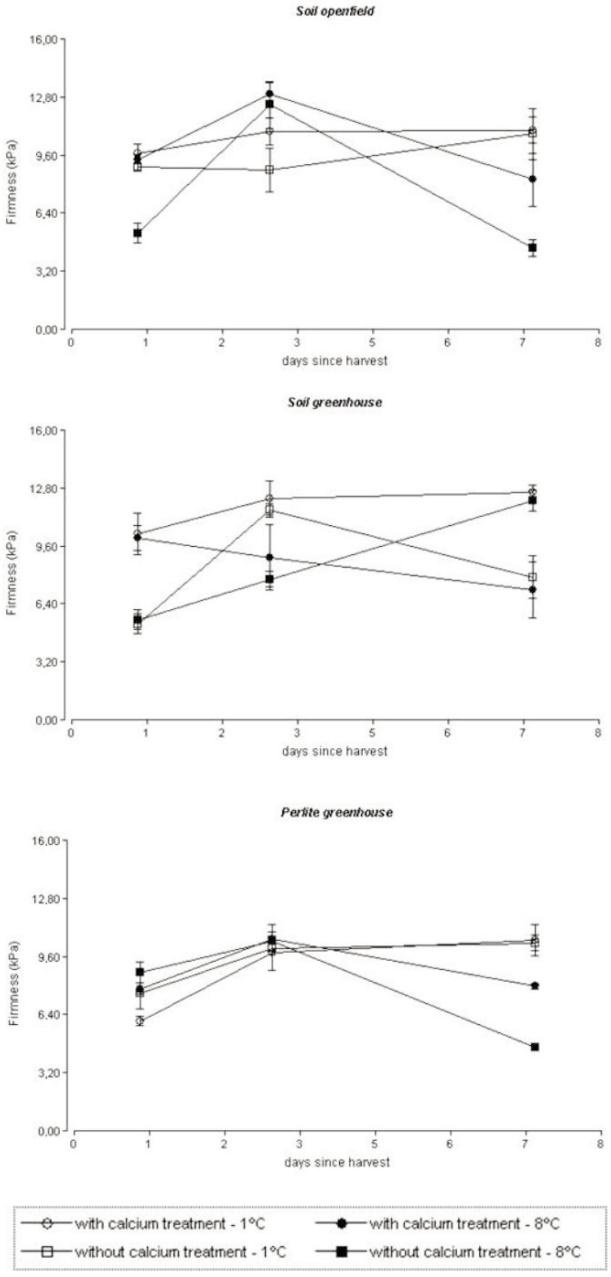

Fig. 4 - Firmness (kPa) at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures.

Table 2 - Total soluble solids (°Brix) at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures

Growing system	Storage 1°C						Storage 8°C					
	1 day		3 days		7 days		1 day		3 days		7 days	
	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without
Soil open field	8 A bc	10 A d	7.63 AB ab	7.83 AB bc	8 A bc	6.33 A a	8.50 A bc	7.17 A a	9.17 AB cd	12 A e	8 A ab	9 AB cd
Soil greenhouse	8 A bc	7.33 B a	7.75 AB ab	8.67 BC cd	8.17 AB bc	9.33 AB de	8.33 A cd	8.17 BC bc	8.33 B cd	7.33 BC ab	6 B a	8.83 B e
Perlite greenhouse	8 A a	9.5 A bc	8 A a	9.17 CD ab	8 A a	8.83 BC ab	9 AB cd	6.5 AB a	8 BC ab	8.33 CD bc	10.5 C de	7.67 BC ab

Different capital letters indicate differences between row and different small letters indicate differences between columns (p<0.05).

field had higher content of soluble solids. It was only in the open field system that a difference between storage temperatures was found: those stored at 8°C had a 12.5% higher content of total soluble solids.

Calcium lactate immersion affected negatively the total soluble solid content. Similar results were found by other authors with different calcium sources. Singh *et al.* (2007) observed that weekly foliar applications of calcium chloride since flowering decreased total soluble solid content in 10% at harvest. Dunn and Able (2006), as well, found that a calcium deficiency during growth stage increased significantly soluble solids content in fruits.

Ascorbic acid content

In almost all cases, ascorbic acid content (Fig. 5) decreased during storage period. Phillips *et al.* (2016) also observed a decrease in strawberries stored at -1.5°C during 7 days. Only the fruits stored at freezers (-10 to -20°C) and ultra freezers (-55°C) maintained ascorbic acid contents similar to those observed at harvest time. Low temperature storage is a key factor to maintain ascorbic acid content during postharvest (Lee and Kader, 2000).

An interaction was observed between calcium treatment and storage temperature (p=0.0026). Untreated fruits stored at 1°C had 26% more ascorbic acid compared to the others. Many authors recognize that a calcium treatment at harvest enhances ascorbic acid content in fruit mainly due to an increase in fruit firmness (Aghdam *et al.*, 2013). Nevertheless, other authors did not find differences between calcium treatment and the control (Shaffie *et al.*, 2010). Regardless the calcium treatment, temperature was the key factor to maintain ascorbic acid content in strawberries.

Fruits grown in perlite had a 49% higher content compared to those grown at soil (both open field and greenhouse). These results are in agreement with de data reported by Treftz and Omaye (2015), who observed that soilless grown strawberries had a 74% higher content of ascorbic acid compared to soil grown fruits. For other fruits, the results were contradictory: Isabelle *et al.* (2010) observed higher ascorbic acid content in pepper and Özcelik and Akilli (1999) in tomato. However, Gruda (2005) didn´t find differences in strawberry.

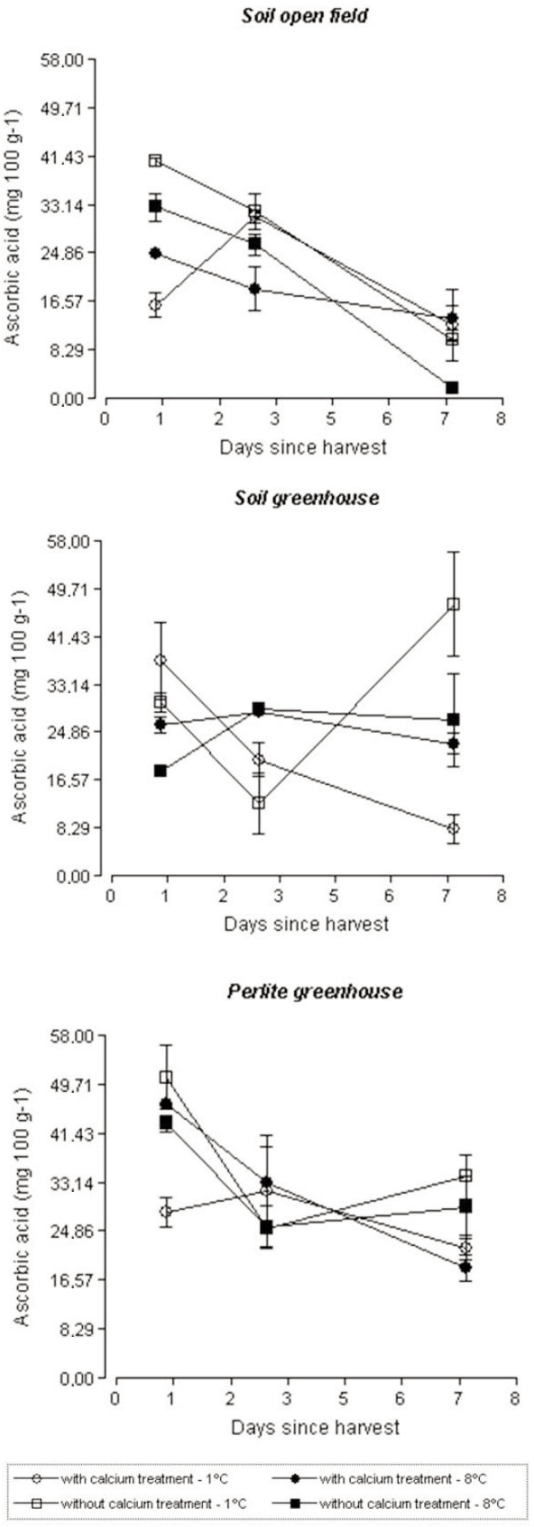

Fig. 5 - Ascorbic acid content (mg 100 g^{-1}) at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures.

Antioxidant capacity

Several interactions were observed for antioxidant capacity. Untreated and greenhouse soil grown fruits presented 13% higher antioxidant capacity (p=0,0003) (Table 3). Wang and Zheng (2001) observed that strawberries grown under higher temperature (day and night) had higher antioxidant capacity, as it was observed in this work, where differences of temperatures between greenhouse and

Table 3 - Antioxidant capacity (mg of ascorbic acid 100 g^{-1} of fresh weight) at 1, 3 and 7 days of postharvest of strawberries grown in different growing systems, calcium lactate treatment and two storage temperatures

Growing system	Storage 1°C				Storage 8°C			
	1 day		7 days		1 day		7 days	
	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without	Ca with	Ca without
Soil open field	150.6 A a	213.1 B ab	232.2 C bc	173.85 AB ab	176.2 AB ab	173.65 AB ab	193.1 AB ab	175.8 AB ab
Soil greenhouse	170.55 AB ab	230.1 C bc	171.05 AB ab	234.65 C bc	157.65 A a	146.7 A a	190.3 AB ab	189.8 AB ab
Perlite	173.15 AB ab	220.55 B b	174.5 AB ab	148.3 A a	162.55 AB ab	174.55 AB ab	201.3 B ab	146.75 A a

Different capital letters indicate differences between row and different small letters indicate differences between columns ($p<0.05$).

open field reached 7°C.

As well, untreated fruits stored at 1°C had 10% higher antioxidant capacity ($p=0.0295$). Many authors, as described in 3.7, explain that a calcium treatment enhances ascorbic acid content (and consequently antioxidant capacity) due to an increase in cell wall firmness. Nevertheless, other authors express that a disruption in cell wall composition increases antioxidant capacity, but this increase is different depending the product (Reyes *et al.*, 2006). With respect to storage temperature, Shin *et al.* (2008) found significant differences since the 12th day.

Different capital letters indicate differences between columns and different small letters indicate differences between rows ($p< 0.05$).

Yield in the growing systems

Although yield was not an objective of this work, we observed that the perlite system had 35% and 17% higher yield than soil in greenhouse and soil in open field systems, respectively.

4. Conclusions

Strawberry is highly accepted by consumers but its postharvest quality rapidly declines. A calcium lactate treatment can increase principally strawberry's firmness. This, in addition to storage temperature and growing system can increase postharvest shelf life of fruits.

In this research, the calcium lactate treatment had a positive effect on fruit firmness, especially in those grown in open field and greenhouse soil. Furthermore, treated fruits had less respiration during storage time and there was no significant difference between storage temperatures in fruits treated with calcium lactate. Equilibrium modified atmosphere was not established.

The other variables were not affected or had a negative response to calcium lactate treatment. Weight loss and organoleptic quality (except visual quality) were better in fruits grown in open field soil, regardless calcium treatment and storage temperature. On the other hand, nutritional quality was better in untreated fruits and stored at 1°C.

In conclusion, even though temperature is substantial to maintain fruit quality at postharvest, a calcium lactate treatment could be useful to improve strawberry (cv. Camarosa) quality during transport and commercialization and decrease incidence of postharvest diseases.

Acknowledgements

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