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Effect of calcium salts and antioxidant treatment on the storage quality of fresh-cut conference pears

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Abstract

The use of calcium chloride and calcium lactate solutions has been shown to be a suitable alternative to chlorine in order to maintain the shelf-life of fresh-cut fruit and vegetables. The objective of this research was to evaluate the effects of calcium chloride or calcium lactate at different concentrations (1, 2, or 4 %, w/v) on the quality and nutritional parameters of whole 'Conference' pears. In addition, the effect of those salts and antioxidant solution (2 % ascorbic acid, 2 % sodium citrate and 1 % calcium chloride) on the quality and sensory attributes of fresh-cut pears was studied. The results showed that the application of calcium salts had no effect on the antioxidant activity (AA), vitamin C content, total phenolic content (TPC), PME and PG activities and calcium content. However, whole pears treated with calcium chloride 1 % showed higher values of AA and TPC than those treated with 2 %. The use of calcium chloride at 1 % combined with antioxidant solution could be a promising preservative method for fresh-cut pears.

Keywords: fresh-cut pear, postharvest, minimal processing, calcium salts, quality

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Introduction

The consumption trend of fruits and vegetables has changed nowadays, mainly due to an increase in consumers demanding not only freshness and healthiness but also convenience (Qadri et al., 2015). Over the last decade, a large number of fresh-cut fruits and vegetables which satisfy those requirements have been developed and are currently being commercialized all around the world. The United Fresh Produce Association defines fresh-cut produce as any fruit or vegetable or any combination thereof that has been physically altered but remains in a state of freshness. In Europe, fresh-cut fruits represent approximately 10 % of the total fresh-cut market volume and their market has grown over the last years especially in countries like Germany and Spain (Baselice et al., 2017). The 'Conference' pear (*Pyrus communis* L cv. Conference) is a Spanish variety recognized as a Protected Denomination Origin fruit. Its production reached 355,410 tons in 2015, 39.5 % of which were produced in Lleida, Spain (MAGRAMA, 2017). The main characteristics of these pears are their low protein, lipid, and glucose content and high levels of other sugars such as fructose, sorbitol, and sucrose. What is more, they are rich in vitamins, minerals, and antioxidant compounds (Colás-Medà et al., 2017) and several studies have suggested their potential for being used in the fresh-cut industry (Colás-Medà et al., 2017; Iglesias et al., 2017, 2018).

Minimal processing includes operations such as cutting, dicing, washing, decontamination, and packaging (Graça et al., 2017). These processes can promote enzymatic browning, softening, tissue degradation (Colás-Medà et al., 2017), decrease in nutritional value, presence of off-flavors or microbiological spoilage (Ma et al., 2017).

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Calcium plays an important role in maintaining the quality of fruit (mainly firmness) and it is well known to protect the integrity of cell membranes by reducing membrane permeability (Kou et al., 2015), pectin solubilization, and matrix glycan breakdown (Belge et al., 2017) and by delaying membrane lipid catabolism (Manganaris et al., 2007). Besides that, calcium treatment has been suggested to be one of the most effective treatments to reduce the activities of pectin methyl esterase (PME, EC 3.1.1.11) and polygalacturonase (PG, EC 3.2.2.67) (Kou et al., 2015). PME and PG perform a coordinated action because the PG acts on the demethylated substrate produced by the activity of the PME (Alandes et al., 2009). The effect of calcium on the firmness of fruit consists of the activation of PME by binding itself to the enzyme as a cofactor (Alandes et al., 2009) and with the interaction with pectin to produce calcium pectate (Ayón-Reyna et al., 2017). Calcium pectate increases the rigidity of the middle lamella and retards PG activity (Aguayo et al., 2008). Calcium chloride has been traditionally used in pre-harvest, post-harvest, and fresh-cut fruit, but currently other calcium salts such as calcium lactate and calcium propionate are also being evaluated as alternative calcium sources (Manganaris et al., 2005). One of the advantages of these salts is that they are more bioavailable than inorganic salts (Hernández-Muñoz et al., 2006) and previous studies have suggested that calcium lactate can reduce bitterness and off-flavors (Rico et al., 2007). Besides firmness, browning is a limiting factor in the shelf-life of fresh-cut fruits. Enzymatic browning is caused mainly by the actions of polyphenol oxidase (PPO, EC 1.10.3.2) and peroxidase (POD, EC 1.11.1.7) (Garcia Loredo et al., 2013). The prevention of softening and enzymatic browning is usually carried out by dipping the minimally processed fruit or vegetable in ascorbic acid, which acts as a reducing agent, in combination with other organic acids or calcium salts (Garcia Loredo et al., 2013; Giacalone and Chiabrando, 2013).

One of the aims of the present study was to assess the effect of different concentrations of calcium chloride and calcium lactate on the antioxidant potential and on the content of calcium, vitamin C (VCC), and total phenols (TPC) of 'Conference' pears. The effect of these salts on the activity of the PME and PG was also evaluated. Furthermore, the current paper also studied the combined effect of calcium salts and an antioxidant solution on key quality parameters such as color, firmness, sweetness or visual appearance of minimally processed pears over an 11-day storage period.

Methods and materials

Chemicals and reagents

Methanol, iron (III) chloride 6-hydrate, sodium hypochlorite, citric acid, and sodium citrate were obtained from Panreac (Barcelona, Spain). 2,2-diphenyl-1-picylhydrazyl (DPPH), L-ascorbic acid, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), sodium carbonate, gallic acid, calcium chloride, polygalacturonic acid, boric acid, 2-cyano-acetamide, and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, potassium dihydrogen phosphate, and Folin-Ciocalteu's reagent were obtained from VWR (Llinars del Vallès, Spain). Ascorbic acid was purchased from Qemical (Barcelona, Spain). Calcium was obtained from Scharlab (Barcelona, Spain). All chemicals and reagents were of analytical grade.

Plant material: Post-harvest calcium treatment and storage conditions

'Conference' pears were purchased from local packinghouses in Lleida, Spain. After selection for uniformity of size, firmness, and color (data not shown) and freedom from defects, the samples were divided into seven lots, one for each water or calcium treatment. All treatments included immersion of the whole fruit in either T0 (control, tap water), T1 (1 % w/v calcium chloride), T2 (2 % w/v calcium chloride), T3 (4 % w/v calcium chloride), T4 (1 % w/v calcium lactate), T5 (2 % w/v calcium lactate), or T6 (4 % w/v calcium lactate) at 25 ºC during 10 min. The fruits were drained at room temperature for 1 h before storage at 0.0 ± 0.5 ºC for 21 d. After storage, approximately half of the samples were frozen using liquid nitrogen, milled, and further stored at -80 ºC until further use.

Assessment of antioxidant activity

Antioxidant activity was determined using two different methods, $DPPH^{\cdot}$ scavenging and FRAP assays. The extraction and the determination procedures were carried out following the method described by Plaza et al. (2016). The results were expressed as μmol of ascorbic acid equivalents per 100 g.

Determination of the total phenolic content (TPC)

The extraction and determination of TPC were determined by the Folin Ciocalteu method following the modifications described by Altisent et al. (2014). The results were expressed as mg of gallic acid equivalent per 100 g.

Determination of the vitamin C content (VCC)

Total vitamin C content (ascorbic acid and dehydroascorbic acid) was determined in triplicate by high performance liquid chromatography (HPLC) using a Waters 717 plus Autosampler HPLC system (Waters Corp., NJ, USA) coupled to an ultraviolet (UV) detector following the method previously described by Plaza et al. (2016). The results were expressed as mg of ascorbic acid per 100 g.

Enzymatic activity

Pectin methyl esterase (PME) activity assay

PME activity was determined according to the method of Plaza et al. (2003) with some modifications. Briefly, the enzyme was extracted by homogenization of 5 g of sample with 10 mL of 0.2 mol L^{-1} sodium phosphate

buffer (pH 7.5) containing 1 mol L^{-1} sodium chloride. The resulting mixture was vigorously shaken for 10 min and centrifuged using a Sigma 3-18KS centrifuge (Osterode am Harz, Germany) at 13,523 × g during 20 min at 4 °C. PME activity was determined titrametrically at 25 ºC by mixing 2 mL of enzymatic extract with 40 mL of a pectin-salt substrate solution (0.35% w/v pectin containing 0.1 mol L^{-1} sodium chloride). The pH of the solution was adjusted to 7.5 using 1 mol L^{-1} sodium hydroxide. After pH adjustment, 0.1 mL of 0.05 mol L^{-1} sodium hydroxide were added and the time needed to recover pH 7.5 was measured. A PME unit was defined on a fresh weight basis as the amount of enzyme required to release 1 μmol of carboxyl groups per second.

Polygalacturonase (PG) activity assay

PG activity was determined following the methodology described by Van linden et al. (2008) with some modifications. Briefly, 6 g of sample were homogenized with 9 mL of cold deionized water. The pH of the mixture was adjusted to pH 3.0 using hydrochloric acid, stirred for 5 min, and centrifuged using a Sigma 3-18KS centrifuge (Osterode am Harz, Germany) at 13,523 × g during 20 min at 4 °C. Reaction mixtures consisted of 350 μL of a 0.2 % (w/v) buffered polygalacturonic acid solution containing 0.04 mol L^{-1} sodium acetate at pH 4.4, and 50 μL of the enzyme extract. Reaction mixtures were incubated at 40 ºC for 10 min. The reaction was stopped by adding 2 mL of cold 0.1 mol L^{-1} borate buffer (pH 9.0). Finally, 0.4 mL of 1 % (w/v) 2-cyano-acetamide solution was added to the reaction mixture which was further incubated at 100 ºC for 10 min and immediately cooled in an ice bath. The absorbance was determined using a GENESYS™ 10S-UV Vis spectrophotometer (Thermo Fisher Scientific, MA, USA) at 276 nm and 22 °C. Enzyme activity was calculated on a fresh weight basis as the release of reducing groups per min.

Determination of calcium content

Determination of calcium was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an ACTIVA-M CCD spectrometer (Horiba Ltd., Kyoto, Japan). The instrument was calibrated using a mono-elemental atomic absorption standard solution for calcium. Sample pretreatment was carried out on a MLS 1200 mega microwave (Milestone, Milan, Italy) with HNO_3 (700 mL L^{-1}), H_2O_2 (300 mL L^{-1}), and nano-pure water. The optic emission was measured at 393 nm and the results were expressed as mg of calcium per kg on a fresh weight basis.

Fruit processing and storage

Samples T0-T6 were stored for 21 d as previously described. After this storage period, pears were cleaned and sanitized by immersion into 0.1 g L^{-1} sodium hypochlorite (pH 6.5 adjusted using citric acid), rinsed, and dried prior to cutting operations. The pears were ripened by storage at 20 ºC until the optimum ripeness stage for processing was reached (Iglesias et al., 2018). Pears were peeled and cut into 10 wedges each using a disinfected handheld apple corer and slicer. Slices of samples T0-T6 were divided into two batches each: one batch was used as a control and was dipped in tap water for 2 min and the other one was treated with an antioxidant solution consisting of 2 % (w/v) ascorbic acid, 2 % (w/v) sodium citrate, and 1 % (w/v) calcium chloride for 2 min. All of the samples were allowed to dry at room conditions and approximately 120 g of the sliced pears were placed in polypropylene trays (120 × 120 × 55 mm) and heat-sealed with a non-peelable polypropylene PP-110 film (ILPRA, Barcelona, Spain) of 64 μm thickness with an oxygen permeability of 110 cm^{-3} m^{-2} d^{-1} atm^{-1} at 23 °C and 90 % of relative humidity. Pear trays were stored at 4 ºC for 11 d and samples were analyzed at days 0, 6, and 11.

Color and texture measurement

Ten color recordings were taken per sample at every sampling point using a Minolta CR-200 colorimeter (Minolta INC, Tokyo, Japan). CIE values were recorded in terms of L^* (lightness), a^* (redness, greenness), and b^* (yellowness/blueness). Calibration was carried out using a standard white tile (Y: 92.5, x: 0.3161, y: 0.3321) provided by the manufacturer and the D65 illuminant, which approximates to daylight. CIE values were used to calculate the browning index (BI) as previously described by Liu et al. (2016). In addition, firmness was determined following the methodology described by Plaza et al. (2016).

Respiration rate

The respiration rate of the minimally processed pears was measured in duplicate following the methodology described by Altisent et al. (2014) using the static method in a closed system with a gas analyser (Checkmate 3, PBI Dansensor, Ringsted, Denmark). The gas analyser was equipped with a solid-state zirconia ion-selective electrode for oxygen determination. To measure carbon dioxide, the gas analyser used a full-scale temperature compensated IR sensor.

Visual appearance and sensory analysis of fresh-cut pears

The visual quality in each replicate was determined by an untrained panel of 10 people, following a previously described methodology (Altisent et al., 2014) based on the following hedonic scale: 9 excellent; 7 very good; 5 good (limit of marketability); 3 fair (limit of usability); and 1 poor (inedible). Based on visual quality results, only pears treated with the antioxidant solution were utilized for sensory evaluation. Consumer tests were carried out following the methodology described by Giné-Bordonaba et al. (2016). Briefly, seven pear slices, one for each treatment (T0-T6) were placed on a white plate and presented to an untrained panel of 30 consumers after 6

and 11 days of cold storage at 4 ºC. All the consumers were regular pear consumers and were the same for all the evaluations assessed. Each piece was identified by a random three-digit code and the order of presentation of the pieces was randomized for each tester. Mineral water was used as a palate cleaner between tastings. Each consumer tested all the samples and was asked to indicate his or her opinion on the overall acceptability of the product using a 9-point hedonic scale (from 1: dislike extremely to 9: like extremely). Sweetness, crunchiness, and firmness were also evaluated with a 5-point hedonic scale (from 1: no perception to 5: strong perception) for each attribute considered. The samples could be re-tasted as often as desired.

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using JMP 8 software (SAS Institute Inc., Cary, NC, USA). The normality of the data and equality of the variances were tested using the Shapiro-Wilk W and Levene tests, respectively. In case of non-normality or unequal variances, the non-parametric equivalents (Wilcoxon/Kruskal-Wallis Tests) were used. Significant disparities were calculated using one-way analysis of variance (ANOVA) and post-hoc HSD Tukey and Student's t-test were used to check the differences. The criterion for statistical significance was $p < 0.05$.

Results and discussion

Effect of calcium treatments and low temperature storage on fruit quality

The current study evaluated the effect of calcium treatment on the physical and nutritional quality of pears (Figure 1). The antioxidant potential was assessed using both, the FRAP and the $DPPH^{\cdot}$ radical scavenging activity (Figure 1A and 1B). Calcium treatment of fruits resulted in increased antioxidant activity in other fruits including apples (Aguayo et al., 2015) and cherries (Aghdam et al., 2013) previously. Results obtained in the current study suggested that the use of calcium salts did not affect the antioxidant potential of the samples when compared to the control. However, the use of calcium chloride at a concentration of 1 % (w/v; T1) resulted in increased antioxidant activity of pears when compared to the same salt at a concentration of 2 % (w/v; T2) ($p < 0.05$).

The current study also assessed the effects of different post-harvest calcium treatments on the VCC content of pears (Figure 1C). A recently published study by Aguayo et al. (2015) reported a 5-fold higher ascorbic acid content in apples after they had been dipped in calcium ascorbate at a concentration of 6% (w/v). The results obtained herein suggest that this trend can also be observed after treatment using calcium chloride and calcium lactate, although the differences observed herein were not statistically significant ($p < 0.05$).

In addition, the results obtained in the current study suggested that postharvest application of calcium chloride and calcium lactate did not affect the TPC of the samples when compared to the control (Figure 1D). However, a significant decrease in the TPC was observed in T2 when compared to T1 ($p < 0.05$). Sample T1 showed the highest TPC which was calculated as 6.99 ± 0.41 mg 100 g^{-1} on a fresh weight basis. Some previously published studies suggested that calcium salts triggered the biosynthetic pathways of phenols and other antioxidant compounds and their utilization resulted in increased TPC in cherries (Aghdam et al., 2013). Moreover, although the TPC was reduced after treatment, Kou et al. (2014) observed an improvement in the retention of phenolic compounds of pears after treatment with calcium chloride (2% w/v, 15 min, 20 °C) when compared to the untreated control. In the current study, the use of calcium chloride and calcium lactate, at the studied concentrations, and after a 21-day storage period did not significantly affect the TPC of pears.

Calcium was suggested to activate enzymes related to the degradation of cell wall components such as PME and PG (Alandes et al., 2009). These enzymes are responsible for fruit softening and their inhibition or control is important in order to increase the products´ shelf-life. Texture is a crucial quality trait orienting consumer choice and, therefore, a key parameter to control. The effect of calcium salts on the activity of both enzymes was evaluated. The results, shown in Figures 1E and 1F, suggested that none of the studied conditions affected the activity of PME. However, both calcium treatments were effective in reducing the activity of PG ($p < 0.05$). Recently, Fortes et al. (2017) suggested that calcium could inhibit PG activity *in vitro* and that the observed reduction in calcium concentration could be linked to an increased PG activity. The reduction in the activity of PG was especially high in T6 (4 % calcium lactate) ($p < 0.05$). Similar results were reported previously by Chuni et al. (2010) who observed that calcium treatment of dragon fruit caused a decrease in the activity of PG when treated at high concentrations. In that study, the authors also observed a decrease in the activity of PME.

Furthermore, the utilization of calcium salts did not affect the calcium content of the sample (Figure 1G). Only the use of calcium chloride at a concentration of 4% (w/v; T3) resulted in increased calcium concentration ($p < 0.05$). Overall, none of the studied treatments affected the antioxidant potential, the VCC, or the TPC of the samples. Although the antioxidant activity and the TPC of pears treated using calcium chloride at a concentration of 1% (w/v; T1) was not significantly different to that of the control (T0), these were higher when compared to pears treated using calcium chloride at a concentration of 2 % (w/v; T2). T1 was also effective at reducing the activity of the enzyme PG ($p < 0.05$).

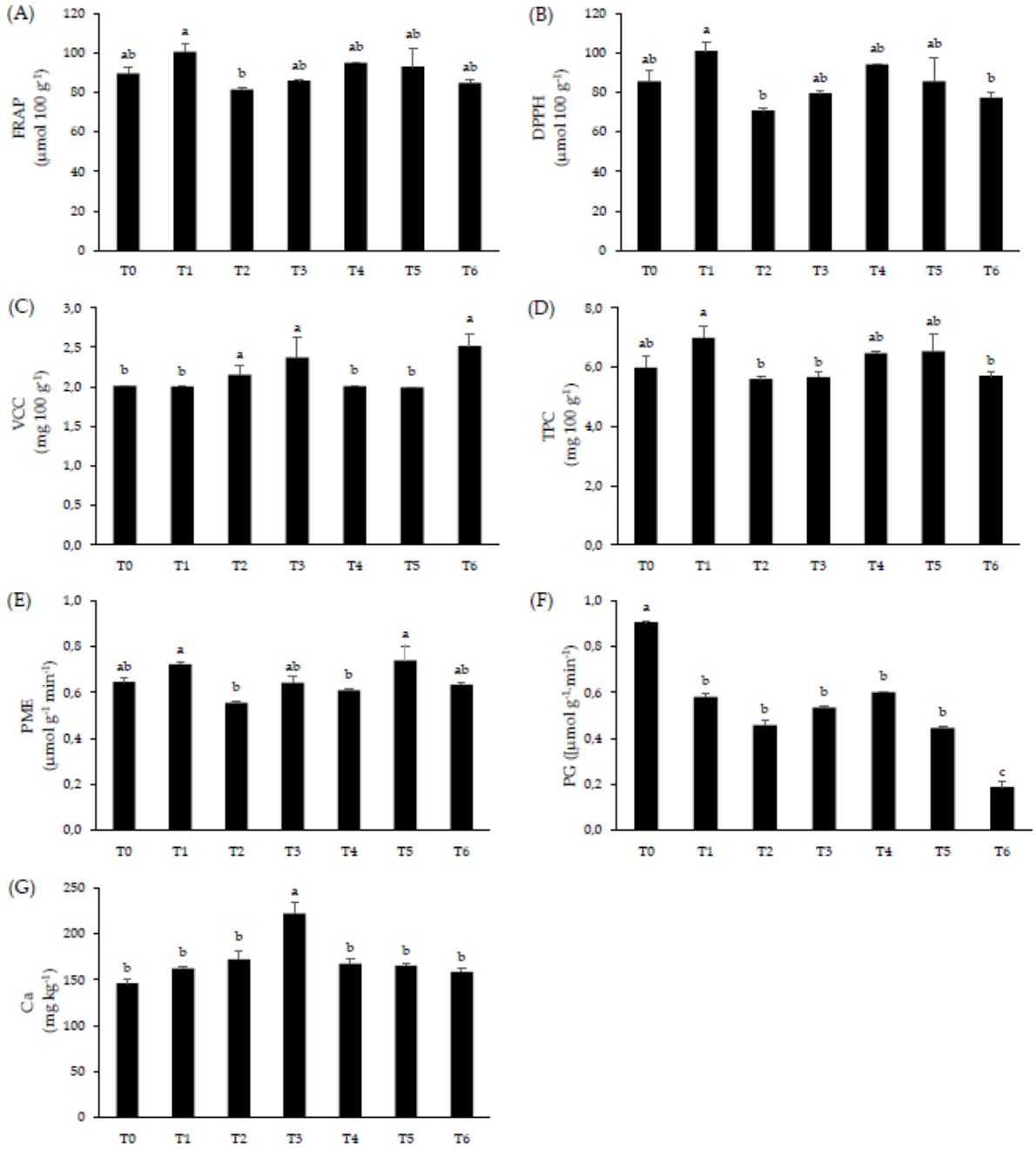

Fig. 1 Antioxidant activity assessed using the (A) FRAP and (B) DPPH· scavenging assays, (C) VCC, (D) TPC, activity of the enzymes (E) PME and (F) PG, and (G) calcium content of 'Conference' pears
Values represent the means of three independent experiments ± SEM. Bars with different letters have mean values that are significantly different ($p < 0.05$)

Effect of postharvest calcium treatments on fresh-cut 'Conference' pears
Respiration rate
Table 1 shows the respiration rate of samples T0-T6 treated with or without antioxidant during an 11-day storage period at 4 ºC. Overall, respiration rate increased significantly after a 6-day of storage period at 4 °C and at day 11 all the oxygen was consumed. Values were higher in those samples with antioxidant treatment when compared to those untreated. There was no clear influence by the calcium treatment on the respiration rate of fresh-cut pears. In addition, for those samples not treated with the antioxidant solution, the utilization of calcium salts significantly increased their respiration rate, except for T2, where no differences were observed ($p < 0.05$). In turn, no differences were observed between the respirations rates of samples treated with the antioxidant solution. The results contrast with previous studies which suggested that postharvest calcium treatments can decrease the metabolic activity of fruit tissues as well as the respiration rate (Tappi et al., 2017).

Table 1 Concentration of oxygen and carbon dioxide in fresh-cut pears during storage

		O_2 [kPa]			CO_2 [kPa]		
Treatment	Antioxidant	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11
T0	No	21.0 ± 0.0 ^{Aa}	6.0 ± 0.3 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	9.7 ± 0.1 ^{Bd}	16.0 ± 0.4 ^{Bc}
	Yes	21.0 ± 0.0 ^{Aa}	2.1 ± 0.6 ^{Ba}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	12.3 ± 0.4 ^{Abc}	18.8 ± 0.3 ^{Ad}
T1	No	21.0 ± 0.0 ^{Aa}	2.1 ± 0.4 ^{Ab}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	12.5 ± 0.3 ^{Abc}	19.1 ± 0.4 ^{Ab}
	Yes	21.0 ± 0.0 ^{Aa}	1.4 ± 0.3 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	12.9 ± 0.2 ^{Ac}	20.1 ± 0.4 ^{Acd}
T2	No	21.0 ± 0.0 ^{Aa}	4.2 ± 0.3 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	11.0 ± 0.1 ^{Bd}	18.9 ± 0.2 ^{Bb}
	Yes	21.0 ± 0.0 ^{Aa}	0.8 ± 0.2 ^{Ba}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	14.2 ± 0.6 ^{Aabc}	21.7 ± 0.8 ^{Abc}
T3	No	21.0 ± 0.0 ^{Aa}	0.7 ± 0.5 ^{Ab}	0.0 ± 0.1 ^{Aa}	0.1 ± 0.4 ^{Aa}	13.9 ± 0.4 ^{Bab}	20.4 ± 0.7 ^{Bb}
	Yes	21.0 ± 0.0 ^{Aa}	0.7 ± 0.1 ^{Aa}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	15.5 ± 0.4 ^{Aab}	23.9 ± 0.1 ^{Ab}
T4	No	21.0 ± 0.0 ^{Aa}	1.4 ± 0.5 ^{Ab}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	13.4 ± 0.3 ^{Bab}	19.8 ± 0.6 ^{Bb}
	Yes	21.0 ± 0.0 ^{Aa}	0.2 ± 0.1 ^{Ba}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	14.6 ± 0.2 ^{Aabc}	21.7 ± 0.4 ^{Abc}
T5	No	21.0 ± 0.0 ^{Aa}	1.7 ± 0.3 ^{Ab}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	13.2 ± 0.3 ^{Bab}	18.9 ± 0.2 ^{Bb}
	Yes	21.0 ± 0.0 ^{Aa}	0.3 ± 0.1 ^{Ba}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	14.5 ± 0.1 ^{Aabc}	22.2 ± 0.5 ^{Abc}
T6	No	21.0 ± 0.0 ^{Aa}	1.3 ± 0.5 ^{Ab}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	14.1 ± 0.7 ^{Ba}	24.5 ± 0.8 ^{Aa}
	Yes	21.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Ba}	0.0 ± 0.0 ^{Aa}	0.0 ± 0.0 ^{Aa}	17.8 ± 0.7 ^{Aa}	27.2 ± 1.1 ^{Aa}

Capital letters indicate significant differences between samples treated with or without antioxidant for each calcium treatment at each sampling point. Lower-case letters indicate significant differences between calcium treatments at each sampling date ($p < 0.05$).

Color and texture

Table 2 lists the *L**, *a**, and *b** values as well as the BI and firmness for each sample. Overall, the BI was lower, and firmness was higher in those samples treated with antioxidant solution when compared to those left untreated. For example, the BI at day 11 of sample T1 treated with the antioxidant solution was significantly lower when compared to that of the untreated sample ($p < 0.05$). The antioxidant solution contained 2 % (w/v) ascorbic acid, which is widely used to avoid enzymatic browning during the storage of fruits. For example, Gorny et al. (2002) demonstrated that immersion of fresh-cut 'Barlett' pears in an antioxidant solution which contained 2 % (w/v) ascorbic acid, 1 % (w/v) calcium lactate, and 0.5 % (w/v) cysteine, completely inhibited the surface darkness for up to 8 days at 0 °C. Correspondingly, the authors of that study suggested that a minimum concentration of 2 % (w/v) ascorbic acid and 1 % (w/v) calcium lactate were necessary to inhibit browning. There were no significant differences in the BI values between samples and a clear trend was not observed. T1 showed the lowest values for samples treated with antioxidant solution after 6 and 11-day of storage period at 4 °C. No differences were observed in the BI of T0 treated with or without antioxidant at days 0, 6, and 11. CIE values of all samples treated with the antioxidant solution remained at acceptable levels during the 11-day storage period at 4 °C considering the threshold values of *L** (>38), *a** (<2) and *b** (<17) proposed by Chen et al. (2010). Overall, the use of antioxidant resulted in a decrease of the *a** value at days 6 and 11 for all the studied calcium treatments as well as for the control ($p < 0.05$). In addition to this, dipping pear slices in the antioxidant solution resulted in increased *L** value at day 11 for all the samples treated with calcium ($p < 0.05$). However, although an increase in the *L** value of T0 was also observed at day 11 after usage of antioxidant, this was not statistically significant. Overall, the different calcium treatments did not affect the color of fresh-cut pears and there were no significant differences between the firmness values of fresh-cut pears neither in non-treated nor in those dipped in antioxidant solution (Table 2, $p > 0.05$). However, the use of antioxidant resulted in improved retention of firmness after 11 days of storage in samples T0, T1, T2, T3, and T5 ($p<0.05$). The antioxidant solution utilized in the current study contained 1 % (w/v) calcium chloride which is usually used to maintain the firmness in fresh-cut fruit and vegetables (Toivonen and Brummell, 2008). The observed increase in firmness at day 11 was not observed at day 6 for samples T0 and T2.

Table 2 Colour and texture changes during storage of minimally processed 'Conference' pears (I)

		L*			a*			b*		
Treatment	Antioxidant	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11
T0	No	76.75 ± 0.74 ^{Aa}	75.04 ± 0.56 ^{Aa}	72.83 ± 0.79 ^{Aa}	-2.61 ± 0.21 ^{Aab}	-2.28 ± 0.11 ^{Aab}	-2.12 ± 0.11 ^{Aa}	11.70 ± 0.58 ^{Ba}	14.27 ± 0.41 ^{Ba}	14.63 ± 0.41 ^{Ab}
	Yes	76.59 ± 2.08 ^{Aa}	74.97 ± 2.93 ^{Aa}	74.91 ± 3.63 ^{Aab}	-3.60 ± 0.30 ^{Bb}	-3.05 ± 0.11 ^{Ba}	-2.73 ± 0.10 ^{Ba}	14.03 ± 0.62 ^{Aa}	15.49 ± 0.43 ^{Aab}	14.79 ± 0.41 ^{Ab}
T1	No	75.88 ± 1.36 ^{Aa}	74.53 ± 0.64 ^{Aa}	69.60 ± 1.04 ^{Ba}	-2.52 ± 0.19 ^{Aa}	-2.29 ± 0.14 ^{Aab}	-1.84 ± 0.13 ^{Aa}	13.91 ± 0.58 ^{Aa}	15.72 ± 0.40 ^{Aa}	16.24 ± 0.37 ^{Aab}
	Yes	76.28 ± 1.07 ^{Aa}	75.17 ± 0.58 ^{Aa}	75.17 ± 0.58 ^{Aa}	-2.66 ± 0.16 ^{Aab}	-3.31 ± 0.13 ^{Ba}	-2.97 ± 0.10 ^{Ba}	11.66 ± 0.57 ^{Bab}	14.19 ± 0.46 ^{Ba}	14.40 ± 0.39 ^{Bb}
T2	No	76.39 ± 0.58 ^{Ba}	73.07 ± 0.89 ^{Aa}	72.38 ± 0.76 ^{Aa}	-2.46 ± 0.14 ^{Aa}	-2.30 ± 0.28 ^{Aab}	-1.99 ± 0.12 ^{Aa}	12.79 ± 0.83 ^{Aa}	16.59 ± 0.64 ^{Aa}	16.27 ± 0.36 ^{Aab}
	Yes	79.49 ± 0.48 ^{Aa}	74.73 ± 0.64 ^{Aa}	74.28 ± 0.70 ^{Aab}	-2.53 ± 0.14 ^{Aa}	-2.95 ± 0.16 ^{Ba}	-2.69 ± 0.09 ^{Ba}	11.73 ± 0.47 ^{Aab}	14.83 ± 0.42 ^{Aab}	15.44 ± 0.33 ^{Aab}
T3	No	78.45 ± 0.68 ^{Aa}	74.06 ± 0.61 ^{Aa}	69.47 ± 1.06 ^{Ba}	-3.41 ± 0.30 ^{Ab}	-2.28 ± 0.14 ^{Aab}	-1.60 ± 0.16 ^{Aa}	14.23 ± 0.71 ^{Aa}	15.86 ± 0.48 ^{Aa}	17.60 ± 0.57 ^{Aa}
	Yes	75.62 ± 0.74 ^{Ba}	74.10 ± 0.76 ^{Aa}	73.57 ± 0.82 ^{Aab}	-3.17 ± 0.28 ^{Aab}	-2.96 ± 0.11 ^{Ba}	-2.59 ± 0.10 ^{Ba}	12.35 ± 0.62 ^{Aab}	16.01 ± 0.44 ^{Aa}	17.11 ± 0.43 ^{Aa}
T4	No	77.02 ± 0.63 ^{Aa}	73.71 ± 0.63 ^{Ba}	70.94 ± 0.84 ^{Ba}	-2.71 ± 0.15 ^{Aab}	-2.71 ± 0.13 ^{Ab}	-1.99 ± 0.11 ^{Aa}	11.65 ± 0.81 ^{Aa}	16.05 ± 0.34 ^{Aa}	15.38 ± 0.29 ^{Ab}
	Yes	75.66 ± 1.67 ^{Aa}	75.56 ± 0.65 ^{Aa}	74.61 ± 0.56 ^{Aab}	-2.76 ± 0.13 ^{Aab}	-3.09 ± 0.10 ^{Ba}	-2.82 ± 0.09 ^{Ba}	10.06 ± 0.73 ^{Ab}	14.33 ± 0.45 ^{Bab}	15.58 ± 0.39 ^{Aab}
T5	No	76.23 ± 0.87 ^{Aa}	72.45 ± 0.71 ^{Ba}	70.83 ± 0.74 ^{Aa}	-2.38 ± 0.10 ^{Aa}	-1.82 ± 0.15 ^{Aa}	-2.05 ± 0.13 ^{Aa}	12.38 ± 0.46 ^{Aa}	15.91 ± 0.51 ^{Aa}	15.43 ± 0.42 ^{Ab}
	Yes	76.48 ± 0.94 ^{Aa}	75.93 ± 0.66 ^{Aa}	72.61 ± 1.12 ^{Ab}	-3.20 ± 0.23 ^{Bab}	-3.11 ± 0.14 ^{Ba}	-2.97 ± 0.15 ^{Ba}	13.04 ± 1.06 ^{Aab}	14.66 ± 0.46 ^{Aab}	16.01 ± 0.47 ^{Aab}
T6	No	77.49 ± 0.80 ^{Aa}	73.99 ± 0.71 ^{Aa}	71.55 ± 1.03 ^{Ba}	-2.85 ± 0.28 ^{Aab}	-2.16 ± 0.09 ^{Aab}	-2.20 ± 0.17 ^{Aa}	12.69 ± 0.72 ^{Aa}	15.08 ± 0.28 ^{Aa}	15.36 ± 0.48 ^{Ab}
	Yes	76.97 ± 0.78 ^{Aa}	75.63 ± 0.52 ^{Aa}	74.51 ± 0.55 ^{Aab}	-2.65 ± 0.26 ^{Aab}	-2.79 ± 0.08 ^{Ba}	-2.76 ± 0.10 ^{Ba}	12.10 ± 0.82 ^{Aab}	14.24 ± 0.32 ^{Aab}	15.72 ± 0.36 ^{Aab}

Capital letters indicate significant differences between antioxidant and control for each calcium treatment at each sampling date and lower-case letter indicate significant differences between seven calcium treatments for antioxidant or control at each sampling date ($p < 0.05$).

Table 2 Colour and texture changes during storage of minimally processed 'Conference' pears (II)

		BI			Firmness (N)		
Treatment	Antioxidant	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11
T0	No	13.53 ± 0.94 ^{Aa}	18.29 ± 0.78 ^{Aa}	19.81 ± 0.92 ^{Ab}	13.95 ± 0.61 ^{Ba}	12.38 ± 0.80 ^{Aa}	11.24 ± 0.93 ^{Bab}
	Yes	16.03 ± 0.80 ^{Aa}	19.43 ± 0.76 ^{Aab}	18.69 ± 0.75 ^{Abc}	16.75 ± 0.52 ^{Aa}	14.07 ± 0.88 ^{Aa}	17.25 ± 1.31 ^{Aa}
T1	No	17.06 ± 0.77 ^{Aa}	20.73 ± 0.75 ^{Aa}	24.12 ± 1.07 ^{Aab}	16.16 ± 1.10 ^{Aa}	11.59 ± 1.11 ^{Ba}	8.58 ± 0.72 ^{Bb}
	Yes	13.52 ± 0.90 ^{Bab}	17.01 ± 0.71 ^{Ba}	17.48 ± 0.68 ^{Bc}	17.15 ± 1.23 ^{Aa}	15.52 ± 1.12 ^{Aa}	16.27 ± 0.89 ^{Aa}
T2	No	15.35 ± 1.24 ^{Aa}	23.06 ± 1.46 ^{Ba}	22.78 ± 0.86 ^{Aab}	16.03 ± 0.38 ^{Aa}	12.53 ± 0.87 ^{Aa}	10.24 ± 0.66 ^{Bb}
	Yes	13.06 ± 0.71 ^{Aab}	18.58 ± 0.79 ^{Aab}	19.89 ± 0.61 ^{Bbc}	15.62 ± 1.01 ^{Aa}	15.00 ± 1.55 ^{Aa}	13.45 ± 1.08 ^{Aab}
T3	No	16.10 ± 1.09 ^{Aa}	21.18 ± 0.95 ^{Aa}	27.18 ± 1.47 ^{Aa}	14.83 ± 0.50 ^{Aa}	10.49 ± 0.76 ^{Ba}	9.81 ± 0.99 ^{Bb}
	Yes	14.10 ± 1.00 ^{Aab}	20.72 ± 0.82 ^{Aa}	23.17 ± 0.89 ^{Ba}	16.16 ± 1.66 ^{Aa}	13.19 ± 0.65 ^{Aa}	14.57 ± 1.13 ^{Aab}
T4	No	13.32 ± 1.16 ^{Aa}	21.15 ± 0.82 ^{Aa}	21.73 ± 0.74 ^{Ab}	16.31 ± 0.78 ^{Aa}	11.85 ± 1.09 ^{Aa}	9.39 ± 0.87 ^{Ab}
	Yes	10.95 ± 0.95 ^{Ab}	17.47 ± 0.85 ^{Bab}	19.94 ± 0.74 ^{Aabc}	16.08 ± 1.20 ^{Aa}	12.53 ± 0.80 ^{Aa}	11.62 ± 0.90 ^{Ab}
T5	No	14.82 ± 0.70 ^{Aa}	22.47 ± 1.21 ^{Aa}	21.76 ± 0.84 ^{Ab}	16.47 ± 1.36 ^{Aa}	11.36 ± 0.80 ^{Ba}	14.67 ± 0.71 ^{Ba}
	Yes	15.11 ± 1.55 ^{Aab}	17.88 ± 0.88 ^{Bab}	21.34 ± 0.93 ^{Aab}	18.78 ± 0.61 ^{Aa}	15.76 ± 0.76 ^{Aa}	16.94 ± 0.52 ^{Aa}
T6	No	14.62 ± 1.06 ^{Aa}	19.99 ± 0.63 ^{Aa}	21.64 ± 1.30 ^{Ab}	16.21 ± 1.41 ^{Aa}	11.91 ± 1.31 ^{Aa}	11.90 ± 1.11 ^{Aab}
	Yes	14.05 ± 1.14 ^{Aab}	17.48 ± 0.61 ^{Bab}	20.21 ± 0.66 ^{Aabc}	16.21 ± 1.51 ^{Aa}	11.82 ± 1.23 ^{Aa}	14.21 ± 0.81 ^{Aab}

Visual appearance

Despite previous studies suggesting that postharvest calcium treatments could reduce the browning of minimally processed fruits, in the current study, the visual appearance of fresh-cut pears, not dipped into the antioxidant solution, was significantly affected (Figure 2). Results suggested that those samples that were not treated with the antioxidant solution were considered inedible by the untrained panel. The antioxidant solution utilized was chosen based on previous studies (data not shown) which suggested that a combination of 2 % (w/v) ascorbic acid, and 2 % (w/v) sodium citrate was the most efficient, from those studied, in maintaining the quality of minimally processed pears during an 11-day storage period at 4 °C. Previously, this antioxidant solution had been efficiently used to maintain the quality of minimally processed pears during storage (Iglesias et al., 2017). No visual differences were detected by consumers between the control T0 and samples T1, T4, T5, and T6 after 6 days of storage. Although the use of 1 % (w/v) calcium chloride did not affect the visual quality of the pears when compared to the control, higher concentrations of this salt (T2 and T3) significantly affected the products visual appearance ($p < 0.05$). However, all of these samples exceeded the limit of marketability and could be therefore commercialized. Gorny et al. (2002) reported visual quality scores under the limit of marketability at day 4 for fresh-cut 'Barlett' pears dipped in either 2 % (w/v) ascorbic acid or 1 % (w/v) calcium lactate (Gorny et al., 2000). The effect of minimal processing on the visual quality of pears can be affected by cultivar (Gorny et al., 2000). Iglesias et al. (2018) also reported lower visual quality scores, but although the pears used in that study were 'Conference' pears, these were stored at higher temperatures.

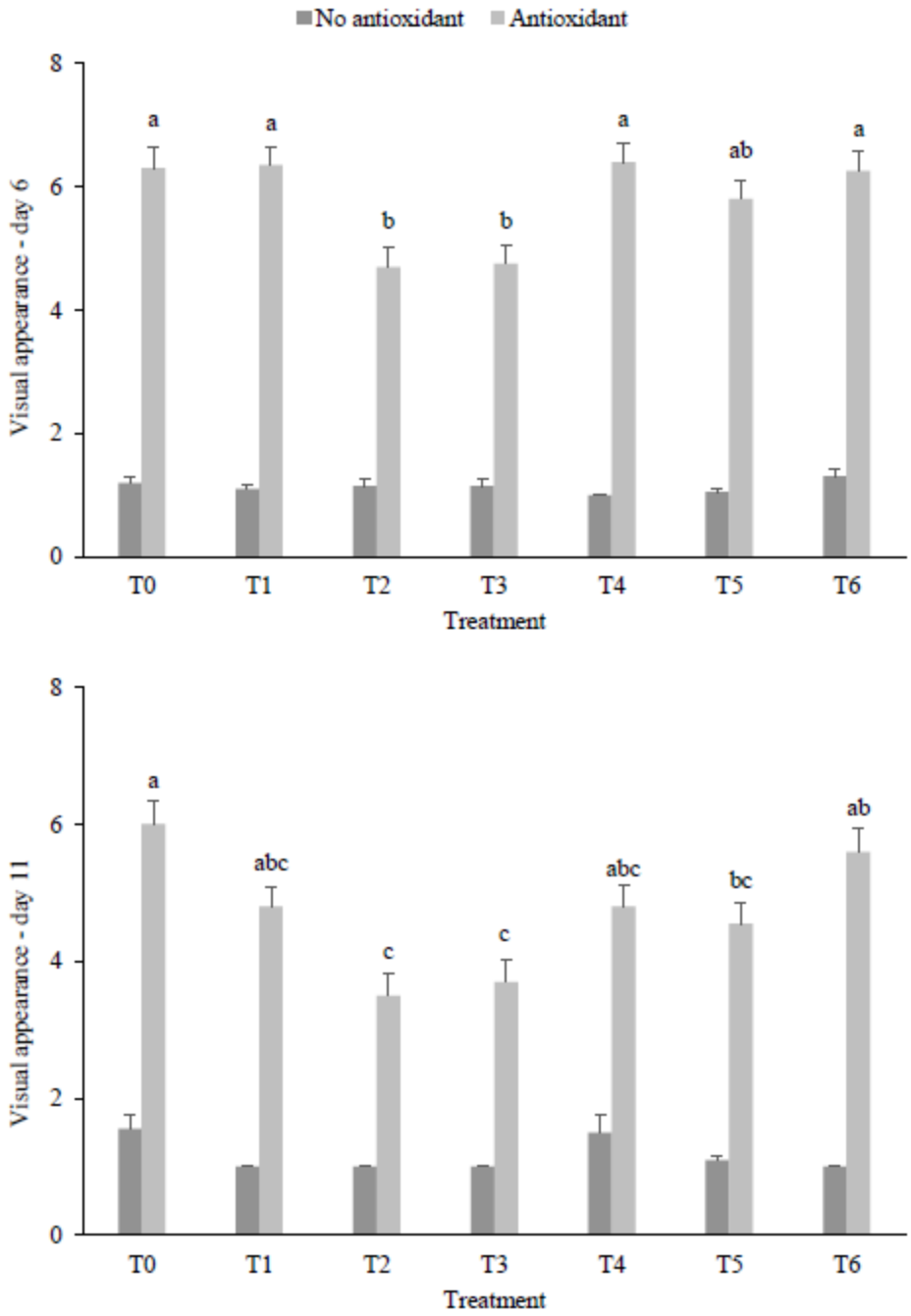

Fig. 2 Visual appearance of fresh-cut pears with and without antioxidant treatment after 6 (A) and 11 days (B) of storage at 4 °C

Fruits were either untreated (dark grey bars) or treated with antioxidant solution (mid-grey bars). Values represent the means of three independent experiments ± SEM. Bars with different letters have mean values that are significantly different ($p < 0.05$)

Sensory analysis of fresh-cut pears

Calcium post-harvest treatments alone were not enough for maintaining the quality of fresh-cut pears, which were evaluated as inedible by the panel. Therefore, samples that were not dipped into the antioxidant solution were not used for sensory analysis. Table 3 lists the acceptance, firmness, crunchiness, and sweetness scores assigned to each sample on days 0, 6, and 11. Overall, the different calcium treatments did not affect firmness, crunchiness, sweetness, or acceptance of the samples when compared to the control at each sampling point. Sensorial property scores were kept constant during storage. However, the acceptance, firmness, and crunchiness scores for T3 and the acceptance and sweetness scores for T4 decreased during storage ($p < 0.05$). The results compare well with previous studies which reported that participants of a test panel could not distinguish between pears treated with different calciums and antioxidants (Gorny et al., 2002). Sweetness or acceptance of other fruits such as papaya were not influenced by different calcium treatments either (Udomkun et al., 2014). The utilization of calcium salts namely calcium chloride and calcium lactate did not affect the sensorial properties of 'Conference' pears including firmness, crunchiness, sweetness, or their overall acceptance.

Table 3 Sensory evaluation of fresh-cut 'Conference' pears during refrigerated storage

	Acceptance			Firmness			Crunchiness			Sweetness		
Treatment	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11	Day 0	Day 6	Day 11
T0	6.0 ± 0.3 ^{Aa}	5.4 ± 0.2 ^{Aa}	5.2 ± 0.3 ^{Aa}	3.7 ± 0.1 ^{Aa}	3.5 ± 0.1 ^{Aa}	3.5 ± 0.1 ^{Aa}	3.9 ± 0.2 ^{Aa}	3.7 ± 0.1 ^{Aa}	3.5 ± 0.2 ^{Aa}	2.7 ± 0.1 ^{Ba}	2.7 ± 0.1 ^{Aa}	2.7 ± 0.2 ^{Aa}
T1	6.0 ± 0.3 ^{Aa}	5.4 ± 0.3 ^{Aa}	5.2 ± 0.3 ^{Aa}	3.9 ± 0.1 ^{Aa}	3.7 ± 0.1 ^{Aa}	3.8 ± 0.1 ^{Aa}	3.9 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	2.8 ± 0.2 ^{ABa}	3.0 ± 0.1 ^{Aa}	2.7 ± 0.1 ^{Aa}
T2	6.5 ± 0.3 ^{Aa}	4.9 ± 0.3 ^{Ab}	5.4 ± 0.3 ^{Ab}	3.7 ± 0.2 ^{Aa}	3.4 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.6 ± 0.2 ^{Aa}	3.1 ± 0.2 ^{ABa}	3.6 ± 0.1 ^{Aa}	2.8 ± 0.2 ^{ABa}	2.7 ± 0.2 ^{Aa}	2.7 ± 0.2 ^{Aa}
T3	6.8 ± 0.2 ^{Aa}	5.5 ± 0.3 ^{Ab}	5.7 ± 0.2 ^{Ab}	3.7 ± 0.1 ^{Aa}	3.1 ± 0.1 ^{Ab}	3.4 ± 0.1 ^{Aa}	3.4 ± 0.1 ^{Aa}	2.8 ± 0.2 ^{Bb}	3.2 ± 0.1 ^{Aab}	3.3 ± 0.1 ^{ABa}	3.1 ± 0.2 ^{Aa}	2.9 ± 0.1 ^{Aa}
T4	6.8 ± 0.3 ^{Aa}	5.2 ± 0.3 ^{Ab}	4.9 ± 0.3 ^{Ab}	3.7 ± 0.1 ^{Aa}	3.3 ± 0.1 ^{Aa}	3.3 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.1 ± 0.2 ^{ABa}	3.2 ± 0.1 ^{Aa}	3.5 ± 0.1 ^{Aa}	2.8 ± 0.1 ^{Ab}	3.1 ± 0.2 ^{Aab}
T5	6.1 ± 0.2 ^{Aa}	5.6 ± 0.2 ^{Aa}	5.2 ± 0.3 ^{Aa}	3.8 ± 0.1 ^{Aa}	3.4 ± 0.1 ^{Aa}	3.4 ± 0.1 ^{Aa}	3.7 ± 0.2 ^{Aa}	3.4 ± 0.1 ^{ABa}	3.4 ± 0.1 ^{Aa}	2.9 ± 0.2 ^{ABa}	2.9 ± 0.1 ^{Aa}	2.6 ± 0.1 ^{Aa}
T6	6.3 ± 0.2 ^{Aa}	5.5 ± 0.2 ^{Aa}	5.5 ± 0.3 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.5 ± 0.1 ^{Aa}	3.6 ± 0.1 ^{Aa}	3.5 ± 0.1 ^{ABa}	3.5 ± 0.1 ^{Aa}	3.1 ± 0.1 ^{ABa}	3.0 ± 0.1 ^{Aa}	3.0 ± 0.2 ^{Aa}

Capital letters indicate significant differences between treatments at each storage time and lower-case letters indicate differences between sampling days for the same treatment ($p < 0.05$).

Conclusions

The effect of calcium chloride and calcium lactate on the quality and nutritional parameters of whole and fresh-cut 'Conference' pears was studied. Overall, the application of calcium salts (chloride or lactate) at different concentrations (1, 2 or 4 %) had no effect on antioxidant activity, vitamin C content, total phenolic content, PME and PG enzymes activities and calcium content of whole pears. The results obtained herein suggested that whole pears treated with calcium chloride at 1 % (w/v) may present higher values of antioxidant activity and total phenolic content when compared to those obtained using higher calcium concentrations. Equally, the application of those calcium salts had no effect on the respiration rates, color parameters, firmness values, visual quality and sensorial characteristics of fresh-cut pears. Fresh-cut pears not dipped in antioxidant solution were not edible for consumers. It could be remarkable that the oxygen concentration values decreased until values close to 0 after 6 and 11-day of storage time at 4 °C. Therefore, postharvest treatment with calcium chloride at 1 %, dipping treatment with antioxidant solution (2 % ascorbic acid, 2 % sodium citrate and 1 % calcium chloride) with minimal processing and storage for 6 d at 4 °C could be the most suitable combination to maintain the quality of fresh-cut 'Conference' pears.

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Author Contribution Statement

Lorena Zudaire, Inmaculada Viñas, Tomas Lafarga, Lucia Plaza, Gemma Echeverria, Gloria Bobo, Rosa Altisent and Ingrid Aguiló-Aguayo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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