

# Effect of calcium and anti-browning agents on total phenols and antioxidant capability of 'Packham's Triumph' pears packed in modified atmosphere

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## Abstract

The effect of calcium propionate (PCa) 1% p/v, ethylenediaminetetraacetic acid (EDTA) 0.1% p/v, cysteine (CIS) 0.5% p/v and citric acid (AC) 0.5% p/v were evaluated on the total phenol content, antioxidant capability and respiration rate of 'Packham's Triumph' pear wedges, packed in modified atmosphere (MA, 8-11% O<sub>2</sub> and 14-16% CO<sub>2</sub> after 6 days of storage) and stored at 5°C for 8 days. The treatments were control (washed with water at 5°C, MA), PCa + EDTA + CIS + AC (MA), and PCa + EDTA + CIS (MA). The evaluations were performed on days 1, 3 and 8 after processing. The respiration rate of the control samples was 42% lower than the pear wedges treated with PCa and anti-browning agents (10-12 mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) during 8 days of storage. On days 3 and 8, the pear wedges treated with PCa + EDTA + CIS + AC and PCa + EDTA + CIS showed respiration rates 55% higher than the control samples. On the other hand, the total phenol content was preserved along the storage period, even though it was 27% lower than the content in peeled fresh fruit (265 mg 100 g<sub>fw</sub><sup>-1</sup>). At the end of the storage period, the PCa + EDTA + CIS showed the highest phenolic content (214 mg 100 g<sub>fw</sub><sup>-1</sup>), while it was lower in the other treatments (146 mg 100 g<sub>fw</sub><sup>-1</sup>). According to the antioxidant capability, non-significantly statistical differences were shown throughout shelf-life, except those wedges treated with PCa + EDTA + CIS whose antioxidant capability was increased by 67%. After the minimally processing operations chemical agent immersions and MA packaging, the total phenol content was diminished compared to the initial fresh flesh fruit. In addition, MA could have a high impact on gas atmosphere inside the packages and preservation of the functional quality of 'Packham's Triumph' pear wedges because no differences were detected among treatments.

**Keywords:** calcium propionate, EDTA, citric acid, cysteine, respiration rate, storage period

## INTRODUCTION

The demand of fresh-cut products has increased due to their convenience, functional and freshness characteristics. However, the operations necessary for their production alter the cell integrity and cause stress in tissues (Beltrán et al., 2005; Martín-Diana et al., 2007). One of the biggest challenges facing the production of fresh-cut fruit is the combination of different preservation factors in order to generate safe products and delay the enzymatic browning reactions to guarantee the sensory quality expected by consumers (Trujillo et al., 2001). Various authors have suggested that modified atmosphere (MA) packaging combined with anti-browning agents and calcium salts preserve the quality of fresh-cut pears (Rosen and Kader, 1989; Gorny et al., 1998; Sapers and Miller, 1998; Gorny et al., 2000; Oms-Oliu et al., 2007).

Sulphites have been widely used to control enzymatic browning in some fresh-cut products such as potatoes. Also, they have been used as a bleaching agent and to control microbial growth. However, sulphites have negative effects on the health of consumers due to them causing severe allergenic reactions (Dong et al., 2000). For this reason, the use of anti-browning agents such as cysteine, EDTA (ethylenediaminetetraacetic acid), ascorbic acid, citric acid and others have been widely studied as an alternative to sulphites (Jiang and



Fu, 1998).

On the other hand, the application of calcium salts has also been studied in various species such as melon, pear and strawberry (Rosen and Kader, 1989; Gorny et al., 1998; Saftner et al., 2003; Aguayo et al., 2008) in order to maintain fresh-cut fruit firmness due to calcium ions binding to free carboxylic groups of pectin chain, increasing the cell wall rigidity (Martín-Diana et al., 2007).

Besides the benefits of calcium in plant tissues, it also contributes to the reduction of browning and microbial growth. As an anti-browning agent it strengthens cell walls and membranes, which avoids the release of enzymes and substrates of the cut surfaces (Rosen and Kader, 1989). Gorny et al. (1998) reported that an application of 1% calcium chloride combined with 2% ascorbic acid reduced enzymatic browning in the tissue of fresh-cut pears. Calcium propionate is widely used as an antimicrobial food additive and it has been shown that treatments at low concentrations do not produce either off flavors or softening (Saftner et al., 2003).

In the same way, the shelf-life of fresh-cut products can be extended by the modification of the internal atmosphere of packages, which is based in the natural interaction between the respiration rate of the product and gas transfer inside the package to generate an atmosphere rich in carbon dioxide and poor in oxygen (Mahajan et al., 2007). Then, this modified atmosphere (MA) can reduce the ethylene production, respiration rate, browning and firmness loss of the product (Oms-Oliu et al., 2007) and preserve the freshness and other quality aspects of fresh-cut fruits as it has been widely studied (Soliva-Fortuny et al., 2007).

Despite fruit and vegetables being rich in natural antioxidants, their consumption has been related to the prevention of cancer and other non-transmissible chronic diseases. Many studies have demonstrated that the antioxidant capability of fresh fruit and vegetables comes from flavonoids and other phenolic compounds, whose biological activity is based on their capacity to reduce the oxidative damage and to catch reactive oxygen species (ROS) (Havsteen, 2002). So, the assessment of changes affecting these compounds with different postharvest treatments is of high interest (Block et al., 1992; Liu et al., 2000). According to Gil et al. (2007) processing has a non-significant effect on the antioxidant compounds of fresh fruit and vegetables. In addition, Bottino et al. (2009) reported that phenolic compounds and the vitamin C content of fresh-cut spinach did not experience changes after processing and 72 h later at cold storage, while Piga et al. (2003) did not observe a reduction of the phenolic and ascorbic acid content of cactus fruit after 9 d of storage at 4°C. However, an optimum storage atmosphere is one of the keys to preserve the sensorial and functional quality and microbiological safety of fresh-cut products (Gil et al., 2007).

The aim of this work was to determine the combined effect of anti-browning agents, calcium salts and MA packaging on the respiration rate, phenolic content and antioxidant capability of fresh-cut pears.

## **MATERIALS AND METHODS**

'Packham's Triumph' pears were provided free of defects by a commercial fresh fruit industry (Kiwi del Sur Ltda, Curicó, Chile). The pears were transported to the laboratory of the Center of Postharvest Studies at the Faculty of Agricultural Sciences, University of Chile, where they were stored at 0±1°C until they were processed.

Minimal processing was conducted at a low temperature (5±0.5°C) in order to minimize respiration and delay the deterioration processes such as browning and softening in the fresh-cut products. The whole fruit was washed with cold water at 5°C for 5 min. Then, the pears were peeled, cored and cut with a sharp stainless steel knife into 6-8 wedges. Subsequently, they were treated by immersions in combined solutions of calcium propionate (PCa) 1% p/v (Granotec, Chile), ethylenediaminetetraacetic acid (EDTA) 0.1% p/v (Sigma-Aldrich, USA), cysteine (CIS) 0.5% p/v (Granotec, Chile) and citric acid (AC) 0.5% p/v (Sigma-Aldrich, USA) and water (as control) for 5 min at 5°C.

Once, the wedges were treated with the combined solutions (PCa + EDTA + CIS + AC and, PCa + EDTA + CIS) around 80 g of fresh-cut pear was packaged in 18×16 cm PD-900

plastic bags (CRYOVAC, Sealed Air Corporation, Chile) with a gas permeability of 3000 mL m<sup>-2</sup> d<sup>-1</sup> O<sub>2</sub> and 9800 mL m<sup>-2</sup> d<sup>-1</sup> CO<sub>2</sub> at 23°C, 1 atm and they were mechanically sealed (Plastic Film Sealer, USA) and stored at 5°C for 8 days.

At the first, sixth and eighth day of storage, three samples of each treatment were randomly selected for the analyses of the respiration rate, gas measurement and bioactive compound concentration.

The analyses of the respiration rate were performed on 0.5-L jars with approximately 500 g of fresh-cut pear. The jars were fitted with air-tight lids equipped with a rubber septum. Then, the jars were stored at 5°C to monitor the respiration rate behavior of the fresh-cut pears along the shelf-life period. During the analysis, a 10 mL gas sample was taken from the headspace of every jar at the designated sampling time (1-1.5 h). Subsequently, the sample was injected into a gas chromatograph (GC) HP 5890 series II (Hewlett Packard Co., Rockville, Md., USA) fitted with a 2.4 m × 3 mm Hayesep Q column (Norwalk, Connecticut, USA) and equipped with a thermal conductivity detector (TCD). The injector and oven temperature were set at 50°C, while the detector was set at 200°C. Helium was used as a carrier gas at 50 psi. The respiration rate was expressed in mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>. The GC was calibrated every day with a 5% O<sub>2</sub> and 10% CO<sub>2</sub> gas standard (Indura, Santiago, Chile).

Measurements of the CO<sub>2</sub> and O<sub>2</sub> levels inside the MA packages during storage were performed with a Check Point O<sub>2</sub>/CO<sub>2</sub> instrument (PBI Dansensor, Milano, Italy). The apparatus is based on an electrochemical sensor to record the O<sub>2</sub> content and a mini-IR spectrophotometer to record the CO<sub>2</sub> content in the package (accuracy: 0.1% O<sub>2</sub>; 2% CO<sub>2</sub>). The instrument was calibrated with O<sub>2</sub> and CO<sub>2</sub> air percentages.

The analyses for the total phenol quantification were performed according to Siriphanich and Kader (1985) with some modifications. For the extract preparation 1 g of fresh-cut pear flesh was blended with 9 mL methanol-water solution (1:1) and it was homogenized for 2 min with an Ultra-Turrax (T18 Basic, Germany) at 13500 rpm. Later, it was centrifuged in a Hermle centrifuge (Hermle Labortechnik Z326K, Germany) at 10000 g<sub>N</sub> for 30 min and the supernatant was filtered through a Whatman N° 2 paper (Schleicher and Schuell, England). Then, 500 µL of the extract was mixed and shaken with a tartrate sodium potassium (0.095 mol L<sup>-1</sup>) and sodium carbonate (0.073 mol L<sup>-1</sup>) solution (1:99, v/v) and it was kept at room temperature for 15 min. Subsequently, 1 mL of Folin-Ciocalteu reagent (1:1, v/v) was added to the mixture and it was incubated in the dark for 1 h at room temperature. Finally, its absorbance was read 660 nm in a UV-WIN spectrophotometer (T 70 UV-Vis PG Instrument Ltd, Leicester, UK) and the results were expressed as mg of gallic acid equivalent (GAE) 100 g<sup>-1</sup> of fresh weight.

On the other hand, the antioxidant capability quantification was based on Benzie and Strain (1999) with some modifications. For the sample preparation, 1 g of fresh-cut pear flesh was frozen with liquid nitrogen (Indura, Chile) and it was ground with a mortar to obtain a fine powder, which was mixed with 9 mL of hydroalcoholic/water solution (C<sub>2</sub>H<sub>6</sub>O/H<sub>2</sub>O, 1:1) and centrifuged in a Hermle centrifuge (described above) at 10000 g<sub>N</sub> for 30 min. Then, the supernatant was filtered through Whatman N° 2 paper and 20 µL of the extract was added to 900 µL of the solution (10:1:1) of acetate buffer (300 mmol L<sup>-1</sup> pH 3.5), ferric chloride hexahydrate (20 mmol L<sup>-1</sup> TPTZ (2,4,6-tri(2-pyridyl)-s-triazine)) and chloridric acid 0.2 M (10 mmol L<sup>-1</sup>) and 80 µL of miliQ water. Finally, its absorbance was read at 660 nm in the UV-WIN spectrophotometer. The results were expressed as mg of Trolox 100 g<sup>-1</sup> of fresh weight.

## RESULTS AND DISCUSSION

### Physicochemical characteristics of 'Packham's Triumph' pear before processing

The raw material was stored for 5 months, at -1.5-0.5°C and 90-95% RH conditions. Before processing, the fruit showed a 4.8 kg<sub>f</sub> firmness, and a total soluble solids content (TSS) around 13% (Table 1). Gil and Zoffoli (1989) state that flesh firmness and soluble solids are the main parameters used for the determination of the harvest date of pears, with recommended values of 7.7 kg<sub>f</sub> and 13% TSS, respectively.



Table 1. Physicochemical characteristics of 'Packham's Triumph' pear before processing.

Physicochemical characteristic	Mean	Physicochemical characteristic	Mean
Skin color		Size (mm)	64.1±0.4 <sup>1</sup>
L	62.6±0.3 <sup>1</sup>	Weight (g)	140.5±0.1 <sup>1</sup>
a*	-5.8±0.5 <sup>1</sup>	Firmness (kg <sub>f</sub> )	4.8±0.1 <sup>1</sup>
b*	41.1±0.2 <sup>1</sup>	Total soluble solids (%TSS)	13.7±0.2 <sup>2</sup>
C*	42.9±0.6 <sup>1</sup>	pH	4.1±0.0 <sup>2</sup>
H <sub>ab</sub>	98.9±0.4 <sup>1</sup>	Total acidity (%)	0.3±0.0 <sup>2</sup>
Flesh color			
L	81.5±0.3 <sup>1</sup>		
a*	-2.4±0.4 <sup>1</sup>		
b*	17.9±0.5 <sup>1</sup>		
C*	18.2±0.6 <sup>1</sup>		
H <sub>ab</sub>	97.5±0.5 <sup>1</sup>		

<sup>1</sup>Mean of 24 samples ± standard error.

<sup>2</sup>Mean of 8 samples ± standard error.

On the other hand, the most common values of firmness and soluble solids used by other authors in the minimally processing of pears are in the range of 4.4 to 5 kg<sub>f</sub> (Gorny et al., 1998; Dong et al., 2000) and 12.6-13.2% TSS (Lu et al., 2009) for firmness and soluble solids content, respectively.

### Respiration rate

After processing, the fresh-cut pears showed a respiration rate of ±17.6-18.8 mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> with no significantly statistical differences among treatments. However, 1 day after processing, control showed a 33% lower respiration rate than the samples treated with PCa + EDTA + CIS, while the pear wedges treated with PCa + EDTA + CIS + AC showed a respiration rate around 12.9 mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>. On day 6, the control sample showed the lowest respiration rate (4.8 mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) while the other samples showed respiration rates of ±10.8-13.4 mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>. At the end of the storage period all treatments had a significantly increased respiration rate compared with control (Figure 1).

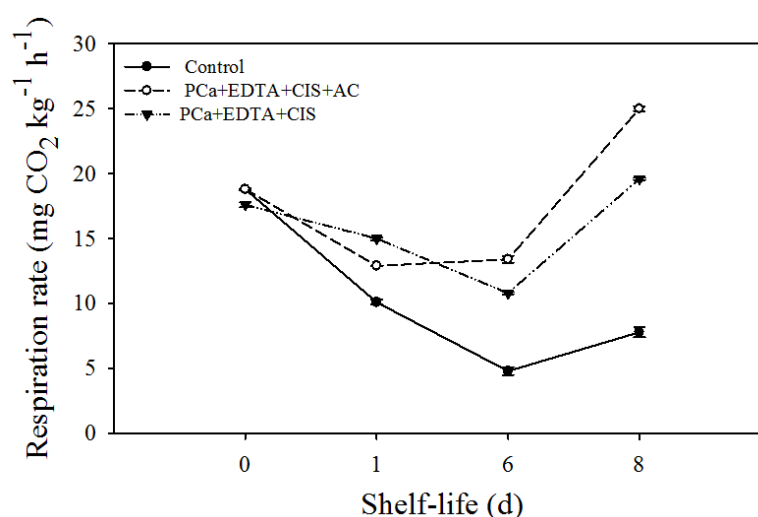


Figure 1. Respiration rate of fresh-cut pears dipped in anti-browning agents, calcium propionate and packed in MA and stored at 5°C for 8 days. Data shown are mean ± error deviation (n=3).

The highest values observed at the beginning of the storage period could be due to the effect of the processing on tissues, which might increase their respiration rate and cause an accelerated consumption of sugars, lipids and organic acids (Beirão-da-Costa et al., 2006). After 1 day of storage the respiration rate of all treatments decreased, which could be due to the tissue recovery of the produce the damaged by the processing (Saftner et al., 2003; Aguayo et al., 2008). During the storage period, the control treatment showed respiration rates lower than the chemical treatments and it could be related to the interference of the anti-browning agents and calcium propionate in the regulation of tissue respiration. For example, citric acid produces a strong reduction of pH and changes in cell membrane permeability, which could affect the carbon dioxide and oxygen emission (Rico et al., 2007). At the end of the storage period, an increase of CO<sub>2</sub> production in all treatments was observed, thus it could be due to the expected deterioration of pear wedges, development senescence and increased microbial growth (Saftner et al., 2003; Benedetti et al., 2008).

### Gas measurements

As it was expected, the use of plastic film generated MA in combination with the respiration rate of pear wedges, where reduced oxygen levels and increased carbon dioxide levels were found (Figure 2). During the storage period, non-significant differences were found among treatments, the O<sub>2</sub> content within packages decreased from 14±0.4 to 3±0.5%, whereas the CO<sub>2</sub> content increased from 8±0.3 to 23±0.4%. After 3 d a steady state of 10±1% O<sub>2</sub> and 15±1.5% CO<sub>2</sub> within MA packages was reached in all treatments. However, on day 6, the O<sub>2</sub> levels decreased (2-3%), while the CO<sub>2</sub> levels increased (20-23%) in all treatments.

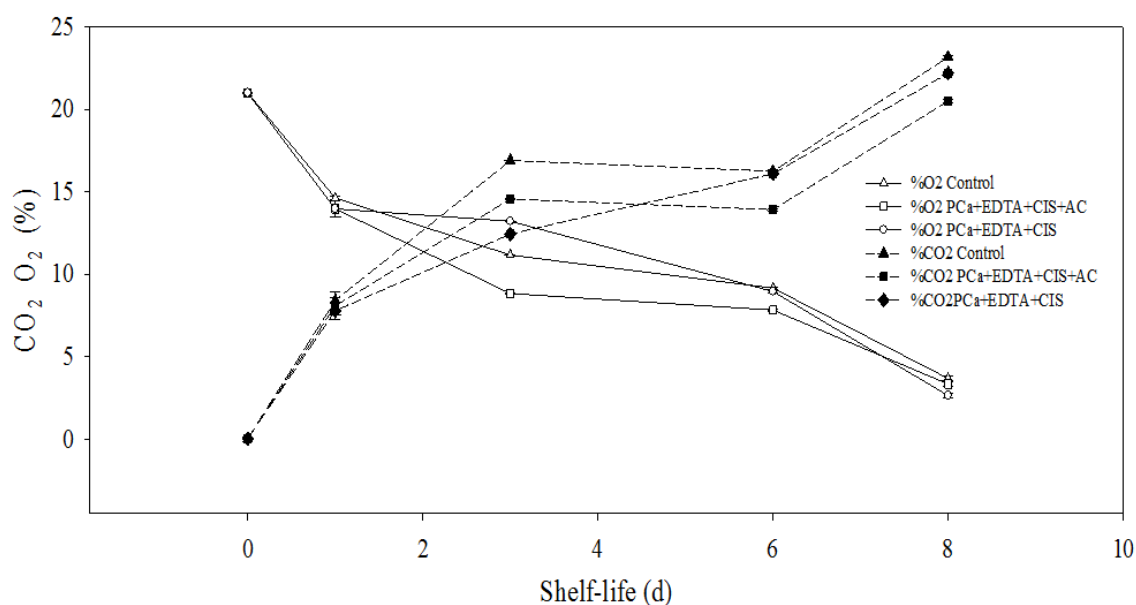


Figure 2. Gas composition changes in fresh-cut pears dipped in anti-browning agents, calcium propionate and packed in MA and stored at 5°C for 8 days. Data shown are mean ± error deviation (n=3).

According to Cantwell and Suslow (2002) the gas composition recommended for fresh-cut pears is 0.5% O<sub>2</sub> and <10% CO<sub>2</sub>; however, the plastic film used in this work did not achieve the recommended composition for this product within the packages, probably due to its high CO<sub>2</sub> permeability. It has been reported that atmospheres with low O<sub>2</sub> (<0.5%) and high CO<sub>2</sub> (>10%) produced off-flavors in fresh-cut pears and accelerate browning in tissues (Gorny et al., 1998; Soliva-Fortuny et al., 2007).

### Total phenol content

After 1 day, non-significant differences were observed among treatments though they showed a 40 mg EAG 100 g<sub>fw</sub><sup>-1</sup> mean reduction as compared with fresh peeled fruit (265 mg EAG 100 g<sub>fw</sub><sup>-1</sup>). At 6 days of storage at 5°C, control showed the lowest total phenol content (160 mg EAG 100 g<sub>fw</sub><sup>-1</sup>) while the pear wedges treated with PCa + EDTA + CIS + AC and PCa + EDTA + CIS showed a content of ±210-220 mg EAG 100 g<sub>fw</sub><sup>-1</sup>, respectively. At the end of the storage period, the control and PCa + EDTA + CIS + AC treatments showed a 160 and 140 mg EAG 100 g<sub>fw</sub><sup>-1</sup> mean content, respectively, while pear wedges treated with PCa + EDTA + CIS presented the highest values (220 mg EAG 100 g<sub>fw</sub><sup>-1</sup>). The effect of the storage time on the total phenols content was only statistically significant in pear wedges treated with PCa + EDTA + CIS + AC, which was decreased by 42% compared with the initial values (Figure 2).

### Antioxidant capability

After 1 day of processing, the highest antioxidant capability was observed in pear wedges treated with PCa + EDTA + CIS (110 mg Trolox 100 g<sub>fw</sub><sup>-1</sup>), followed by PCa + EDTA + CIS + AC (100 mg Trolox 100 g<sub>fw</sub><sup>-1</sup>) and control treatment (50 mg Trolox 100 g<sub>fw</sub><sup>-1</sup>). In addition, a mean reduction around 90 mg Trolox 100 g<sub>fw</sub><sup>-1</sup> was observed when comparing the treatments to peeled fresh fruit (190 mg Trolox 100 g<sub>fw</sub><sup>-1</sup>). After 6 days of storage at 5°C, non-statistically significant differences were found among treatments. At the end of the storage period, control and PCa + EDTA + CIS + AC treatments showed an antioxidant capability of 30 and 60 mg Trolox Trolox 100 g<sub>fw</sub><sup>-1</sup>, respectively, while pear wedges treated with PCa + EDTA + CIS showed the highest antioxidant capability (80 mg Trolox 100 g<sub>fw</sub><sup>-1</sup>). The effect of the storage time was significant only in the pear wedges treated with PCa + EDTA + CIS which was decreased by 20% compared to the initial values (Figure 3).

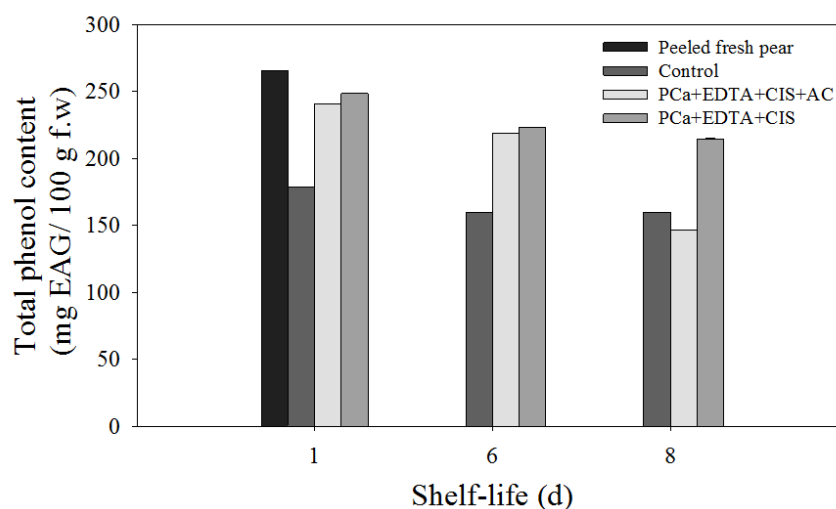


Figure 3. Total phenol content of fresh-cut pears dipped in anti-browning agents, calcium propionate, packed in MA and stored at 5°C for 8 days. Data shown are mean ± standard error deviation (n=3).

The effect of MA packaged on the total phenol content and antioxidant capability could not avoid the reduction when comparing with the initial content of a recent peeled fresh fruit (Figure 4) as was reported by Lee and Kader (2000), who found that that MA (0.5, 2 or 4% O<sub>2</sub>) decreased the antioxidant capability of kiwi slices by around 18%. This trend could be due to the fast oxidation of phenolic compounds in tissue surface, due to their direct interaction with the oxygen inside the package. In addition, the enzymatic oxidation of phenolic compounds, such as chlorogenic acid catalyzed by polyphenol oxydase has been related to the enzymatic browning of fresh-cut pears (Gil et al., 1998). For that reason, active MA with low O<sub>2</sub> levels might be used since the beginning of the storage.

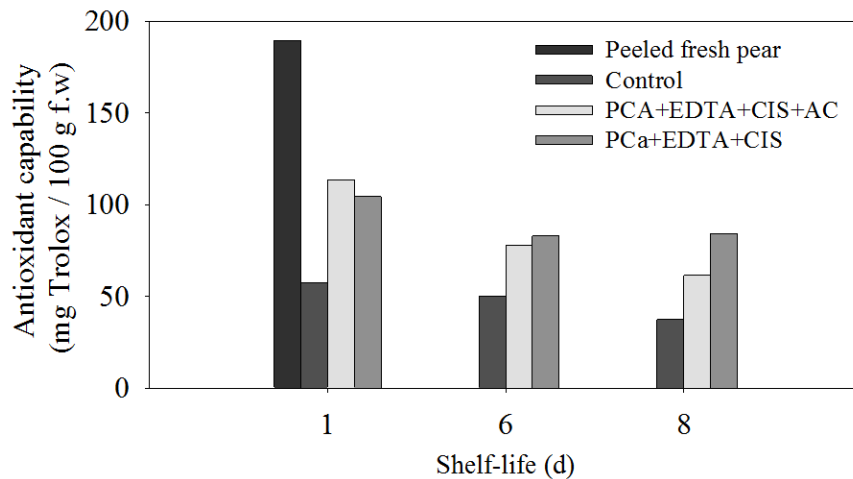


Figure 4. Antioxidant capability of fresh-cut pears dipped in anti-browning agents, calcium propionate, packed in MA and stored at 5°C for 8 days. Data shown are mean  $\pm$  standard error ( $n=3$ ).

## CONCLUSION

The application of anti-browning agents and calcium propionate increased the respiration rate of pear wedges in comparison with the control treatment. However, MA packaging combined with anti-browning agents and calcium propionate could be a tool to preserve the functional quality of fresh-cut products, due to, as it was observed in this study, the treatments applied to fresh-cut pears allowed the total phenol content and antioxidant capability of pear wedges to be preserved along the storage period

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