

Use of xanthan gum and calcium ascorbate to prolong cv. Butirra pear slices shelf life during storage

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: After cut, pear fruit (*Pyrus communis* L.) during shelf life can be subjected to color and flavor changes. To maintain flesh colour and firmness, different technologies could be employed during shelf life, such as chemical, physical and edible coating treatments. In the present study, the effects of two edible coating formulations containing xanthan gum and calcium ascorbate on fresh-cut pear fruit were investigated. After harvest, 200 fruits were cut and coated with Xanthan Gum (XAN) and distilled water or Xanthan gum + Calcium Ascorbate (ASC), respectively, while control (CTR) pear fruit slices were soaked in distilled water and lastly packed in polyethylene terephthalate (PET) packages sealed with a composite film (PP-PET). All samples were stored at $5 \pm 0.5^\circ\text{C}$ with RH 90% for 10 days. Measurements were carried out at 3, 5, 7 and 10 days of storage evaluating visual quality score, browning index, color, total solid soluble content (TSS), flavor, ascorbic acid content and total phenols content. The results showed that ASC treatment was the most efficient treatment in terms of color changes, ascorbic acid content, visual quality score and browning index, until the 7th day of storage. Moreover, ASC treatment reported lower mean values in terms of taste and flavor score if compared to CTR and XAN treatments. Untreated pear slices (CTR) kept good values concerning flavor score until the 3rd day of storage while on the 5th and 7th day off-flavor values were the same as treated samples.

1. Introduction

‘Butirra’ pear fruit is cultivated in southwestern Sicily and belongs to sicilian MIPAAF PATs (traditional agricultural products). After harvest, usually occurring between the second decade of July and the first decade of August, they must be consumed as they are easily rotten and are subjected to fast decay. It’s easy to understand how fresh-cut ‘Butirra’ poor shelf-life is a key barrier to its commercialization especially for the fast weakening of tissues and surface’s browning that happens after cut due to the action of polyphenol oxidase (PPO) (Amiot *et al.*, 1995; Hodges and Toivonen, 2008). Edible coatings are widely employed since they prevent the loss of quality acting like a selective barrier to gas exchanges between

food and external environment. Xanthan gum enhances all these properties and also controls the rheology of the final food product exhibiting pseudo-plastic properties in solutions (Palaniraj and Jayaraman, 2011). As a generally recognized as safe (GRAS) molecule, xanthan gum is an exopolysaccharide produced by the fermentation of a carbohydrate by cultures of *Xanthomonas campestris*. It is then refined by extraction with ethanol or 2-propanol, dried, and powdered (FDA). Calcium ascorbate is the calcium salt of ascorbic acid that is widely used as an antioxidant whose reducing action against quinones and diphenols prevents browning of unprocessed fruit as it only produces colorless derivatives; it is a reducing agent, capable of promoting the chemical reduction of the pigment precursors responsible for browning, acting by reducing o-benzoquinone or dihydroxyphenol or irreversibly inactivating PPO, promotes the regeneration of antioxidants and acts synergistically with complexing agents (Araújo, 2004). Furthermore, this cation can maintain cell wall structure by binding to pectins and forming calcium pectate (Vilas Boas *et al.*, 2009). Calcium stabilizes the membranes and cell walls, preserving their integrity and functionality and protecting them from being cleaved by hydrolytic enzymes that cause fruit softening (Poovaiah, 1986; Vilas Boas *et al.*, 2009). Xanthan gum combined with antioxidant agents had positive effects on the reduction of weight loss and browning, preventing the loss of firmness, and the growth of psychotropic microorganisms, molds and yeasts in minimally processed apples and pears (Sharma and Rao, 2015; Allegra *et al.*, 2022). The aim of the present study was to evaluate the effectiveness of edible coating based on xanthan gum and xanthan gum enriched with calcium ascorbate on fresh-cut cv 'Butirra' pear fruit stored in passive atmosphere.

2. Materials and Methods

The experiment was carried out in 2021. 'Butirra' pear fruit (Quince BA29 rootstock and intermediate 'Butirra Hardy' graft) were harvested during the second week of august in a commercial orchard located in Zafferana Etnea (Catania, Italy), Italy, (730 m above sea level). The soil is a sandy clay loam (63% sand, 19% silt, 18% clay), with pH 6.9 and active carbonates lower than 5%, trees were trained as a free palmette. Fruits were hand-picked at an optimal ripening stage tested with Lugol solution. All trees received the

same conventional cultural cares from planting until the end of the current experiment. After harvest, fruit were cold stored and transported at University of Palermo and stored at $5\pm1^{\circ}\text{C}$ in cold room the night before the analysis.

Experimental design

Two hundred fruit were harvested from 20 trees and brought to the laboratory where they were dipped in chlorinated water (100 ppm of free chlorine) for 360 s to sanitize them. Defective fruit were discarded and the remaining were sorted by firmness ($4.1\pm1\text{ kg/cm}^2$) and average weight ($120\pm 20.2\text{ g}$). Quality indexes were calculated the first day of analysis, particularly, color (CIELab), flesh compactness and total solid soluble content (TSS).

Fruits were selected for weight, maturation index, caliber and absence or presence of defects and sanitized with OxVirin 200 ppm and H_2O by soaking for 30 minutes. Then, they were peeled and cut. Edible coatings were applied by dipping and solutions were formulated as follows:

- i) Control (CTR): fruits were dipped in distilled water and used as control;
- ii) XAN: the solution was made by mixing 3% of xanthan gum in distilled water using a magnetic stirrer;
- iii) ASC: the solution was made by mixing 3% of xanthan gum and 2% of calcium ascorbate in distilled water using a magnetic stirrer.

After treatments, fruit were packed in PET boxes, sealed with a composite PP-PET film and stored at $5\pm1^{\circ}\text{C}$ with 95% relative humidity (RH) for 10 days. Trials were carried out at 3, 5, 7 and 10 days of storage evaluating visual quality score, browning index, color, total solid soluble content (TSS), sensorial analysis, ascorbic acid content and total phenols content.

Weight loss

The following formula was adopted to determine weight loss during storage

$$\text{Weight loss (\%)} = [(W1-W2)/(W1)] \times 100$$

Where $W1$ and $W2$ represent initial weight ($T0$) and measured weight at 3, 5, 7 and 10 days of storage with a precision balance (Gibertini, Italy), respectively. At the beginning of trial period all boxes had homogeneous weight ($100 \pm 2.1\text{ g}$).

Color

Flesh color was measured throughout the experiment on the first day of analysis (0) and on the 3rd,

5th, 7th and 10th day of storage. Color was measured through a portable colorimeter (Chroma Meter CR 400, Konica Minolta Sensing Inc., Tokyo, Japan) equipped with an 8 mm measuring head and a C illuminant (6774 K). The white standard plate of the manufacturer was used for calibration. Chromatic difference (ΔE) was calculated using the following formula to express the magnitude of difference between the non-aged pulp and stored samples:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

All trials were carried out in triplicate and data were reported as $\pm$ mean standard error (SE n=3).

Browning index

Browning index (BI) was determined following the equation of Ruangchakpet and Sajjaanantakul (2007):

$$(BI) = [100 (x - 0.31)] / 0.17$$

where $x = (a^* + 1.75 L^*) / (5.645 L^* + a^* - 0.3012b^*)$.

Firmness

Fruit firmness was tested with a texture analyzer equipped with a 2.5 cm flat-tip (Instron 5564, MA, USA). The maximal force was expressed in kg/cm² and slices were compressed with a speed of 5 mm/s to a depth of 4 mm.

Total solid soluble

Total solid soluble content was determined on pear fruit juice extracted from samples at each storage time using a hand-held refractometer (ATAGO Palette PR-32).

Total phenols content

Total phenols content was quantified according to Sortino *et al.* (2022). 30 grams of fresh tissue for each replication was homogenized with methanol on 1:10 ratio and then filtered through a Whatman grade N.1 filter, the application of reduced pressure allowed the concentration of methanolic extracts and the residue was then suspended in 50% aqueous methanol and used for phenolic content quantification. Phenols content was determined through a spectrophotometrical analysis at the wave length of 700 nm and results were expressed in gallic acid equivalent (mg kg⁻¹ fresh weight).

Ascorbic acid

Ascorbic acid content was analyzed at each sampling date with the Megazyme kit (Bray Business Park, Bray, Co., Wicklow, Ireland) as reported by Allegra *et al.* (2015).

Sensorial analysis

Sensorial analysis was carried out by a panel of 12 specifically trained panelists (Sortino *et al.*, 2017). All samples were subjected to a panel made up of 14 descriptors: external color uniformity (ECU), compactness (COM), pulp color intensity (PCI), odor (O), herbaceous odor (HO), floral odor (FO), sweetness (SW), sourness (S), bitterness (B), juiciness (J), pear flavor (PF), herbaceous (HF) and floral flavor (FF) and overall rating (OR). The graduated scale went from 1 (absence of descriptor) to 9 (descriptor at its fullest intensity). Sensorial analysis was carried out from day 0 to day 10.

Visual quality score

Edible coatings effect on 'Butirra' fresh-cut slices was evaluated at each storage time on six slices used as single replicates, for each treatment. Six trained judges used a list of descriptors wrote down in preliminary meetings. Descriptors involved the medium value of color, integrity and appearance on pear fruit slices as reported by Allegra *et al.* (2022). Descriptors were quantified using a 5 points hedonic scale where 5= very good, 4= good, 3= sufficient (limit of marketability), 2= poor (limit of usability) and 1= very poor (inedible).

CO₂ and O₂ inside packaging

O₂ and CO₂ content inside packages was analyzed at each sampling date using a PBI Dansensor Checkpoint O₂-CO₂ analyzer (Ametek Mocon, MS, USA) equipped with infrared detectors.

Statistical analysis

The experimental design consisted in two treatments and one control, observed at 0, 3, 5, 7 and 10 days at 5°C after treatment. Nine slices were used as single replicates and analyzed at each sampling date. Analysis of variance was applied (Systat 13.0 for Windows was used as statistical software) and the significance of data ($p \leq 0.05$) was evaluated with Tukey's test.

3. Results and Discussion

Total solid soluble content

Our results showed a general increase in TSS during storage; control slices content increased about 29.03% during the 10 days of storage while XAN and ASC treatments scored an increase of 16.30% and 15.45%, respectively. CTR slices increase is probably

due to the greater water loss of the untreated samples, which also results in higher percentages of weight loss (Fig. 1). XAN and ASC treatments were more efficient than CTR in limiting the increase in TSS content, this phenomenon is due to ripening processes that result in the hydrolysis of starch into mono- and disaccharides (Mahajan *et al.*, 2004) and in the activation of respiration processes where sugars are the main substrate used (Dong *et al.*, 2004). Significant differences occurred between treatments on 5th and 7th day while on 10th day XAN and ASC treatment recorded the same value.

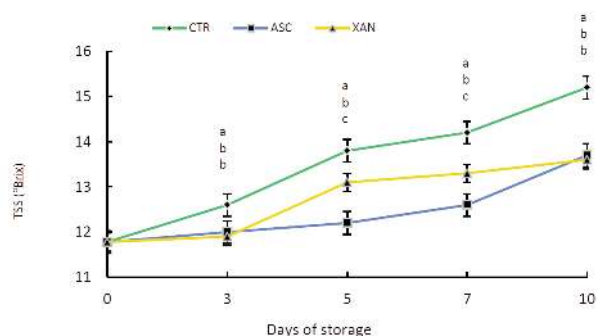


Fig. 1 - Total solid soluble content of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significant differences between treatments. $p < 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

Weight loss and firmness

Weight loss during cold storage showed an increase in all sample slices (Fig. 2). CTR samples showed a higher percentage of weight loss if compared to other treatments. ASC treatment showed lower weight loss than XAN and control pear slices. The effectiveness of ascorbate calcium can be attributed to the ability of calcium to preserve the compactness of cell structures by limiting the action of pectolytic enzymes. In other work on fresh-cut pear the use of calcium ascorbate could be responsible of the maintenance of cellular wall structure since calcium maintains glycosidic bindings stable avoiding the collapse of cellular wall and the subsequent loss of liquids (Akhtar *et al.*, 2010). Grant *et al.* (1973), on the other hand, showed that the maintenance of firmness in calcium-treated fruits may be due to its accumulation in cell walls, which facilitates the crossing of pectic polymers by increasing wall strength and cell cohesion. Obtained results for weight loss are

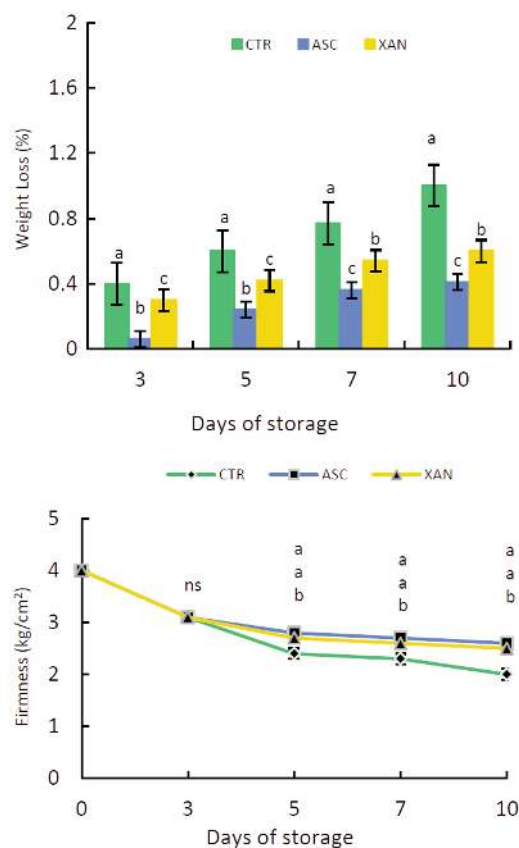


Fig. 2 - Weight loss (%) and firmness (kg/cm²) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. 'Butirra' fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significant differences between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

closely linked, as previously discussed, with TSS content data.

Color changes and browning index

Color monitoring during cold storage showed a change in L*, a* and b* values in 'Butirra' in all treatments (Table 1). A low decrease in L* values occurred in XAN treatment. Color changes are more limited in ASC treated samples.

At the end of cold storage period (T10), ΔE reached its highest in CTR samples and its lowest in ASC treated samples. Untreated 'Butirra' fruit slices recorded the highest browning values at each sampling date, and it began to increase sharply from day five, while XAN and ASC treatments recorded the lowest values. Significant differences occurred between ASC and XAN treatments from 5th day of storage while no significant differences were recorded

Table 1 - Color slices (CIELab index) and color variation (ΔE) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C

Days of storage	Treatments	L*	a*	b*	ΔE
T0	CTR	63.89	-13.57	36.94	-
T3	CTR	64.64 a	-0.32 b	24.30 b	18.33 NS
	ASC	66.27 b	-0.38 b	24.98 b	17.96
	XAN	64.82 a	0.61 a	26.16 a	17.84
T5	CTR	66.97 a	-0.03 c	20.82 NS	27.27 a
	ASC	63.96 b	-0.11 b	19.25	22.23 b
	XAN	57.51 c	0.51 a	19.25	23.50 b
T7	CTR	65.12 a	-0.22 a	17.4 b	28.69 a
	ASC	63.02 b	-0.07 b	16.72 b	24.33 b
	XAN	52.39 c	0.01 b	20.02 a	24.55 b
T10	CTR	45.53 c	-0.02 b	12.20 b	33.66 a
	ASC	62.65 b	0.03b	14.90 a	25.93 b
	XAN	65.24 a	-0.36 a	14.37 a	26.19 b

At each sampling date, different letters indicate significative differences between treatments. NS = not significant. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

ed at 10th day between the two edible coatings formulations (Fig. 3). A similar trend occurred in Sharma and Rao (2015) on fresh-cut pear treated with Xanthan gum for 8 days of cold storage.

Sensorial analysis and visual quality score

Sensorial analysis was carried out at each storage time and at harvest time high values were recorded for all descriptors except for bitterness, sourness, herbaceous and floral odor and flavor. A slower

decrease in treated pear slices was recorded. All treatments recorded higher values than CTR one at each sampling time and no off-flavors or negative descriptors were found in edible coating treated samples until 10th day of storage. In fact, 'Butirra' pear slices treated with ASC and XAN recorded positive values for descriptors concerning sweetness, compactness, external color uniformity, juiciness and pear flavor during all storage time while, on the contrary, CTR samples recorded increasing values of negative descriptors such as bitterness and sourness as early as the 5th day of analysis. Overall rating was quite positive in treated samples until 10th day (Fig. 4).

Visual quality score test enhanced that a decrease in mean values occurred in all treatments registering significant differences since the 3rd day of analysis (Fig. 5). Since day 3, CTR samples recorded mean values under 3 (limit of marketability). Instead, the judges evaluated ASC samples on 10th day at the limit of marketability (score=3). Xanthan coating had a positive effect on visual score of fresh-cut pear (Sharma and Rao, 2015) extending their marketability until the 10th day.

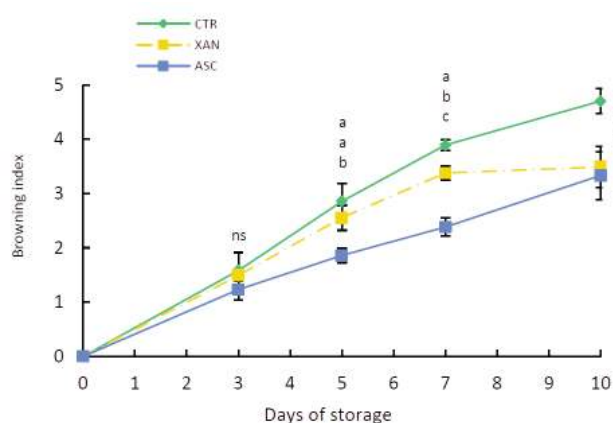


Fig. 3 - Browning index of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

Total phenols and ascorbic acid content

No significant differences were showed between treatments on pear slices ascorbic acid content. During storage, total phenol content increases slowly in both CTR and XAN treatment. After 7 days a sharp increase was observed in treated and untreated samples and significant differences occurred between

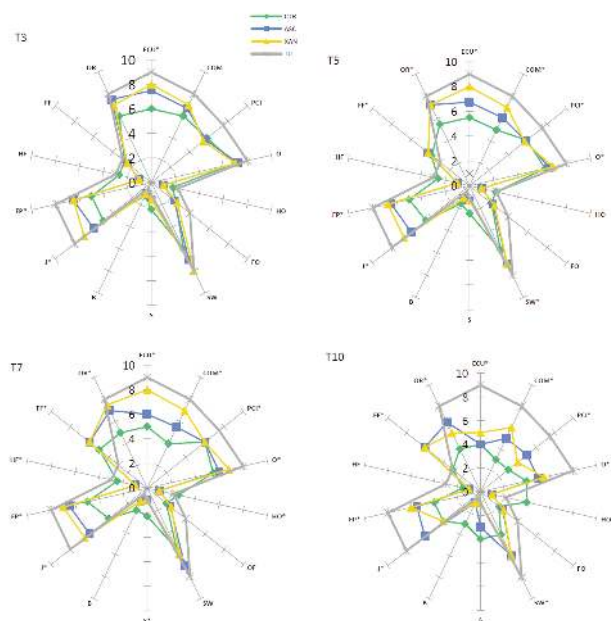


Fig. 4 - Sensorial analysis of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices after cut (0) and at 3, 7 and 10 days of storage at 5°C. At each sampling date, * indicates substantial changes between treatments. At each sampling date, ns indicate no changes between treatments. $p \leq 0.05$ was used in the Tukey's test. Legend: external color uniformity (ECU), compactness (COM), pulp color intensity (PCI), odor (O), herbaceous odor (HO), floral odor (FO), sweetness (SW), sour (S), bitter (B), juiciness (J), pear flavor (PF), herbaceous flavor (HF), floral flavor (FF) and overall rating (O).

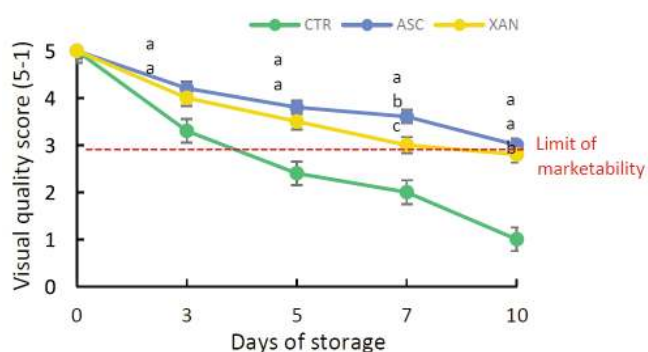


Fig. 5 - Visual quality score of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significant differences between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

ASC and other treatments (Fig. 6). An increase of total phenol content is possible in stress conditions after cutting or in low temperature (Amodio *et al.*, 2014).

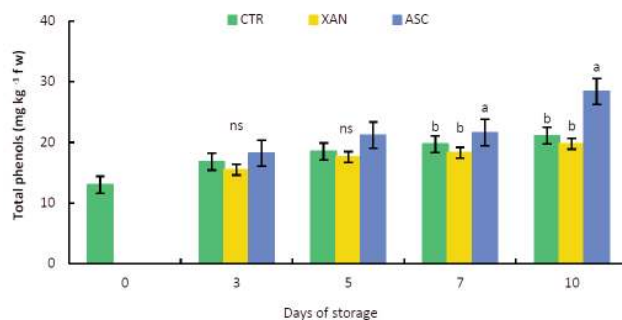


Fig. 6 - Total phenols content ($\text{mg kg}^{-1} \text{fw}$) of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after storage for 3, 5, 7, 10 days at 5°C. At each sampling date, different letters indicate significant differences between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

CO₂ and O₂ inside packaging

A limited O₂ consumption and CO₂ production occurred during storage in both XAN and ASC treatments while higher values were registered in CTR samples (Fig. 7). Significant differences occurred from the 3rd day between treated and untreated samples, particularly, CTR samples registered a value of 1.5 kPa for O₂ and a value of 21 kPa for CO₂ at 10th day of storage. Both XAN and ASC kept the two parameters more stable. Similar trends were observed on fresh-cut peach treated with calcium lactate and ascorbic acid and on breba fig fruit stored in passive atmosphere (Allegra and Colelli, 2015; Allegra *et al.*, 2015).

4. Conclusions

The two different formulations based on calcium ascorbate and Xanthan gum preserved pear slices of 'Butirra' during the 10-day storage at 5°C. Positive effects were observed on browning, weight loss and firmness up to the 10th day, furthermore, the two edible coating formulations preserved the sensory attributes of fresh cut 'Butirra'. Our results showed that Xanthan gum with calcium ascorbate treatment improved the retention of firmness, browning and weight loss than control slices. This result was confirmed by the sensorial analysis in which positive descriptors showed positive values until the 10th day of storage while untreated samples began to develop off-flavor and off-color since day 3.

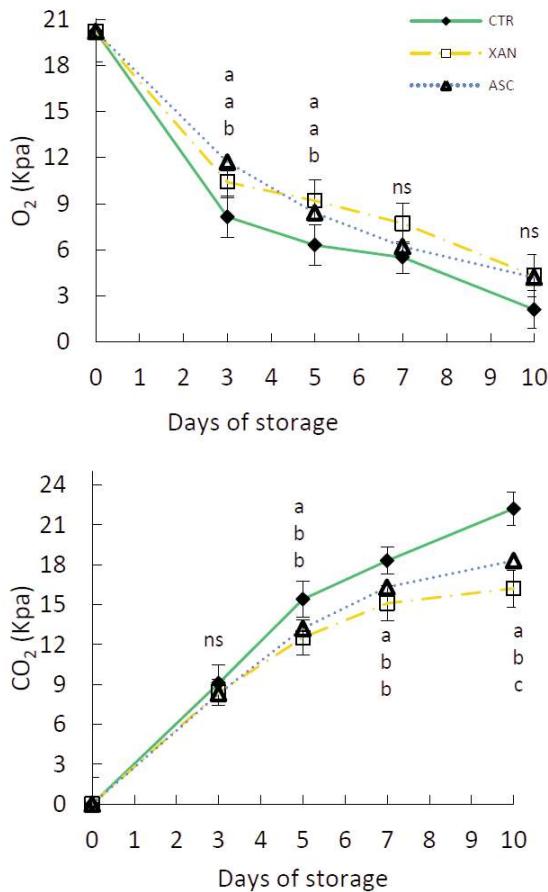


Fig. 7 - O₂ and CO₂ inside packaging of untreated (CTR) and treated with Xanthan gum and Xanthan gum + calcium ascorbate (XAN and ASC) *Pyrus communis* L. cv. Butirra fruit slices at 0 time and after for 3, 5, 7, 10 days of storage at 5°C. At each sampling date, different letters indicate significative differences between treatments. $p \leq 0.05$ was used in the Tukey's test. The data are provided as the mean $\pm$ SE (n = 3).

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