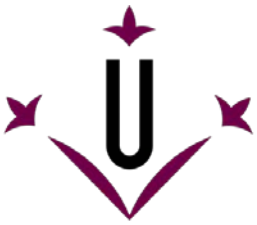

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Development of a fresh-cut product based on pears and the subsequent evaluation of its shelf life under commercial conditions and after a cold chain break.

P. Colás-Medà[1], M. Abadias[2*], R. Altisent[2], I. Alegre[1], L. Plaza[2], V. Gilabert[3], R. Lacomba[3], I. Viñas[1]

*Corresponding author: isabel.abadias@irta.cat

[1] Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio, 25198-Lleida, Spain

[2] IRTA, XaRTA-Postharvest, 25003-Lleida, Spain

[3] Grupo Alimentario Citrus, 46394-Ribarroja de Turia, Spain

Abstract Processing of pears as a fresh-cut product could offer added value and introduce a product into the market that offers greater convenience and health benefits for consumers. Cultivar selection is one of the most important considerations for fresh-cut fruit processing because characteristics such as flesh texture, skin colour, and browning potential can vary greatly among cultivars. Four pear cultivars ('Flor de invierno', 'Passe-Crassane', 'Ercolini' and 'Conference') and four antioxidant treatments, that is, (NS) 50 g L^{-1} NatureSeal® AS1 (Agricoat) solution, (AsAc) 20 g L^{-1} ascorbic acid + 10 g L^{-1} citric acid + 10 g L^{-1} calcium chloride solution, (CaAs) 20 g L^{-1} calcium ascorbate + 10 g L^{-1} calcium chloride solution and (NaAs) 20 g L^{-1} sodium ascorbate + 10 g L^{-1} calcium chloride solution, were tested to obtain a high-quality fresh-cut pear. For the selected cultivar and treatment, the nutritional changes and physicochemical, microbial and sensorial quality were evaluated under conditions that simulated commercial application followed by storage at 4 °C and a simulated cold chain break at 8 °C. The 'Conference' pear was selected as the best cultivar based on its physicochemical characteristics (high levels of soluble solids content and low acidity), low increase in browning index, and visual acceptance after 7 days of storage. The results demonstrated that CaAs maintained the fresh-cut pear quality after 8 days of storage at 4 °C and also after a cold chain break. Furthermore, application of the selected treatment produced an increase in the ascorbic acid content, total phenolic content and antioxidant activity of minimally processed pear samples. These values were reduced during shelf life, but the total phenolic content at the final sampling point was higher than that of fresh-cut pears after processing without treatment.

Keywords: *Minimally processed, variety, anti-browning, total phenolic content, ascorbic acid, antioxidant activity*

1. Introduction

Fruit and vegetables are important components of a healthy diet, and sufficient daily consumption could aid in prevention of major diseases such as cardiovascular diseases and certain cancers. In the last decade, several countries joined to launch international health recommendations that promoted the consumption of 400 to 500 g of fruit and/or vegetables per day, the equivalent of five 80 g servings. This approach to improved consumption was referred to as '5 a day'. Our area (Lleida, Catalonia) of Spain is the country's main producer of pears (176.640 tons produced in 2014), which are primarily commercialized as fresh fruit, and different cultivars such as 'Blanquilla', 'Conference', 'Ercolini', 'Llimonera' and others are grown [1]. Processing of pears as a fresh-cut product could create added value and introduce a product to the market that offers greater convenience and health benefits to consumers. Cultivar selection is one of the most important considerations in fresh-cut fruit processing because characteristics such as flesh texture, skin colour, and browning potential can vary greatly among cultivars [2]. The suitability of different cultivars for processing has been previously studied [2-5], but certain pear cultivars in our area have not been studied. Minimal processing operations damage the tissue integrity of fruit, causing an increase in physiological activity and leading to biochemical changes such as browning, off-flavour development and softening [6]. Enzymatic browning occurs when o-diphenol substrates react with oxygen to generate o-quinones, which subsequently polymerize and result in dark melanins. The oxidative reaction is catalysed by polyphenoloxidase (PPO) [7]. To minimize this visual deterioration, treatments that involve dipping of fruit slices into aqueous solutions containing antioxidants and calcium salts are widely practiced to improve the quality of fresh-cut fruit. A great number of studies have been conducted to avoid browning surfaces on fresh-cut pears using selected reducing agents such as ascorbic acid, 4-hexylresorcinol, cysteine, N-acetylcysteine and sodium eritorbate [3, 8-11]. These acidifying additives have a reduction action against quinones, and diphenol prevent browning of minimally processed fruit because it produces only colourless derivates [3]. Another concern related to extension of shelf life for fresh-cut fruit is softening, which is primarily due to enzymatic degradation of the cell wall, which is mainly composed of cellulose, hemicelluloses and pectins. Calcium salts, and particularly calcium chloride and lactate, are generally used in combination with browning inhibitors as firmness-maintaining agents in a wide range of cultivars of fresh-cut fruit and vegetables [12]. Calcium can interact with the free carboxyl groups liberated by the de-esterification of pectin by pectinmethylesterase (PME) to form insoluble calcium pectates, which strengthen the structure of the cell wall [13].

To develop a fresh-cut pear product, the main considerations are selection of the most appropriate cultivar, stage of ripeness at cutting, choice of the best antioxidant treatment, and selection of adequate packaging. During storage of the packaged product, certain changes occur in the surrounding atmosphere. These changes depend on the respiratory activity of the product, its storage temperature, the permeability of the packaging films and the ratio of the packaging area to the amount of fruit [5, 10, 14]. The low O_2 and/or elevated CO_2 environment generated by modified atmosphere packaging of fresh-cut product can extend the product shelf life by slowing the browning reactions at the cut surfaces, reducing the

rates of product transpiration (water loss) and respiration, and reducing ethylene biosynthesis and action [3, 15]. The aim of this study was to select the best cultivar and antioxidant treatment to obtain a high-quality fresh-cut pear. For the selected cultivar and treatments, the physicochemical quality, nutritional changes, microbial quality and sensorial quality were evaluated at conditions that simulated commercial application at 4 °C and a cold chain break at 8 °C.

2. Materials and methods

2.1 Selection of the most suitable pear cultivar

2.1.1 Fruit and fruit processing

Four pear cultivars ('Flor de invierno', 'Passe-Crassane', 'Ercolini' and 'Conference') were purchased at commercial maturity from commercial orchards in Lleida (Catalonia, Spain). Before processing, the flesh firmness of whole pears from each cultivar was measured on opposite sides of each fruit with a penetrometer (Effegi, Mila, Italy) equipped with a probe 8 mm in diameter. Eight fruits per cultivar were measured, and the results were reported in Newtons (N). Prior to experimental studies, pears were disinfected by immersion in a 0.1 g L^{-1} sodium hypochlorite (NaClO) solution (pH 6.5) for 2 min, rinsed in running tap water and allowed to dry at room temperature. Pears were peeled and cut into 10 wedges using a handheld apple corer and slicer.

2.1.2 Antioxidant treatment

NatureSeal® AS1 was used to select the pear cultivar because its effect was widely studied and is effective in different fresh-cut fruit. Pear wedges were treated by immersion in an antioxidant solution of 50 g L^{-1} NatureSeal® AS1 (NS, Agricoat) (w/v), and distilled water was used as a control (CK). In brief, pear wedges were dipped (1:2 w/v) for 2 min at 150 rpm on an orbital shaker in cold water plus the corresponding treatment. After treatment, the wedges were allowed to dry at room conditions. Fresh-cut pears (120 ± 5 g) were placed in polypropylene terephthalate trays (APET, 375 mL) and sealed with a non-peelable polypropylene terephthalate plastic film (APET-110, ILPRA, Italy) with a thickness of 64 µm and an O_2 permeability of 110 cm^3 m^{-2} d^{-1} atm^{-1} at 23 °C. This packaging was chosen based on a previous short trial. Trays were stored at 5 ± 1 °C, and samples were examined after treatment (0 day) and after 7 days.

2.1.3 Fresh-cut fruit quality evaluation

To determine the most suitable pear cultivar, surface colour, texture, soluble solids content (SSC) and titratable acidity (TA) were assessed after fruit processing . After 7 days of storage at 5 °C, before the quality evaluation, the headspace gas composition was determined using a handheld gas analyser (CheckPoint O_2/CO_2, PBI Dansensor, Denmark) and the visual acceptance was evaluated. Surface colour was determined immediately after that trays were opened. Afterwards the rest of determinations were done.

The visual evaluation of pear wedges from different cultivars and treatments (CK and NS, three trays per treatment) was conducted by an untrained panel using a 9-points hedonic scale: 9=excellent; 7=very good; 5=good (limit of

marketability); 3=fair (limit of usability); and 1=poor (inedible) [18]. An average was obtained for each cultivar and treatment after 7 days of storage.

After fruit processing and after 7 days of storage, the surface colour of pear wedges was determined using a chromameter (model CR-200 Minolta, Minolta Inc., Tokyo, Japan). Colour readings were measured on both sides of five pear wedges (n= 10) per cultivar and treatment on the day of processing (0 day), and five wedges per tray were examined in each cultivar and treatment (n= 30) after storage. Data were obtained as CIELab* values but results were expressed as the hue angle (h°= arctan (b*/a*)) and the browning index (BI) value (BI= 100*(x-0.31)/0.172, where x= (a* + 1.75 L*)/ (5.645 L* + a* - 3.012 b*)) according to Buera et al. [17].

Prior the texture evaluation, the pear wedges were cut into 20 x 20 mm pieces. The texture of fresh-cut pears was evaluated after processing and after storage according to Altisent et al. [16] parameters. Five texture measurements per cultivar and treatment were performed after processing (0 day), and three measurements per tray were performed per cultivar and treatment (n= 9) after 7 days of storage.

At each sampling point, the pear wedges were squeezed, and the soluble solids content (SSC) was determined using a handheld refractometer at 20 °C (Atago CO., LTD, Japan). Three measurements were collected per treatment (one measurement per tray), and the results were reported as percentage of soluble solids in fruit juice (%).To measure titratable acidity (TA), triplicate samples of 10 mL of extracted fruit were diluted with 10 mL of distilled water, and 2 drops of phenolphthalein solution 1 % RV (Panreac, Barcelona, Spain) were added. The solutions were titrated with sodium hydroxide solution (NaOH, 0.1 mol L^{-1}) until a colour change of the pH indicator occurred. Three measurements were collected per treatment. The results were calculated in terms of g of malic acid per litre of solution.

2.2 Antioxidant selection

2.2.1 Fruit processing

Selection of antioxidant treatment was performed with ‘Conference’ pears, which were used at their optimum ripeness stage (44 ± 3.2 N), according to Soliva-Fortuny et al. [19] and our previous experiences. Flesh firmness was measured as described previously. To obtain this ripeness stage, pears were stored at 20 °C until they reached the desired firmness. Pears were subsequently subjected to the processing operations described above.

2.2.2 Antioxidant treatment

In order to evaluate an alternative antioxidant treatment to control browning in fresh-cut pears, the following treatments were tested: (AsAc) 20 g L^{-1} (w/v) ascorbic acid + 10 g L^{-1} (w/v) citric acid + 10 g L^{-1} (w/v) calcium chloride solution, (CaAs) 20 g L^{-1} (w/v) calcium ascorbate + 10 g L^{-1} (w/v) calcium chloride solution and (NaAs) 20 g L^{-1} (w/v) sodium ascorbate + 10 g L^{-1} (w/v) calcium chloride solution. Fresh-cut pears without antioxidant treatment (distilled water, CK) and treated with the commercial NS product (50 g L^{-1} NatureSeal® AS1) were included as controls to evaluate the effectiveness of the proposed antioxidant combinations. The concentrations of the antioxidant agents were

chosen in accordance to current bibliography. All chemical products evaluated in this study are currently approved for use as food additives in minimally processed fruit [20]. The antioxidant applications in the 'Conference' pear wedges were conducted by immersion, as described above. Subsequently, fresh-cut pears (120 ± 5 g) were placed in the same APET trays and sealed.

Trays were stored at 5 ± 1 °C, and samples were examined on the day of preparation (0 day) and after 7 (three trays) and 14 days of storage (three trays). In addition, headspace gas composition, visual quality, colour, texture, SSC and TA were assessed as previously described. Headspace gas composition and visual quality were only determined after the storage periods.

2.3 Semi-commercial assay

Based on previous results, an assay simulating commercial conditions was performed with the 'Conference' pear and CaAs (20 g L^{-1} (w/v) calcium ascorbate + 10 g L^{-1} (w/v) calcium chloride) as the antioxidant. Water was used as a control (CK). The pears were processed, treated with antioxidant solution, and packaged as described above. Three trays per each treatment were examined at 0 day and after 3 days of storage at 4 ± 1 °C. The remainder of the samples were divided into two lots with one stored at 8 ± 1 °C until 8 days (simulated cold chain break) to simulate more realistic conditions during transport and in the refrigerated display window, and the other was maintained at 4 ± 1 °C until 8 days (realistic cold chain conditions). After 3 days of storage at 4 °C, after 8 days of storage at 4 °C (realistic cold chain conditions) and after 8 days of storage under simulated cold chain break (3 days at 4 °C plus 5 days at 8 °C), the same evaluations were performed as in the previous steps: headspace gas composition, visual quality, colour, texture, SSC and TA. In addition, nutritional analysis, microbial quality and consumer acceptability were evaluated.

2.3.1 Nutritional evaluation: Bioactive compounds and antioxidant activity

Furthermore, the semi-commercial assay ascorbic acid content, total phenolic content and antioxidant activity of samples were determinate at processing day (0 day) and after 3 and 8 days of storage (at 4 or 8 °C).

Determination of the ascorbic acid content was performed as described by Altisent et al. [16] with minor modifications. The results were expressed as grams of ascorbic acid per kg of fresh weight.

For antioxidant activity and total phenolic content determination, 6 g of frozen sample was homogenized with 20 mL of methanol 70 %. The mixture was centrifuged, filtered and adjusted to 25 mL with extraction solution (30 g L^{-1} meta-phosphoric acid + 80 mL L^{-1} acetic acid). With the extracts obtained, the antioxidant activity was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging assay following the procedure described previously (Altisent et al., 2014) with minor modifications. The total phenolic content was determined by the Folin-Ciocalteu method with certain modifications. The antioxidant activity results and the total phenolic content results were expressed as mmoles of ascorbic acid equivalents per kg of fresh weight and as grams of gallic acid per kg of fresh weight, respectively.

2.3.2 Microbial quality

The microbial quality of minimally processed 'Conference' pears treated with antioxidant solutions was evaluated during the shelf life. At each sampling time, 25 g of each tray were diluted in 225 mL of buffer peptone water (BPW, Oxoid) and homogenized in a stomacher blender (IUL, Masticator, Spain) at 250 impact s^{-1} for 90 s. Serial dilutions of the suspension were conducted in sterile buffer peptone water (BPW, Oxoid) and analysed for psychrotrophic microorganisms (PM), yeasts and moulds (YM), and lactic acid bacteria (LAB) according to standard (ISO) methodologies (ISO 17410:2001, ISO 21527-1:2008, ISO 15214:1998, respectively). In brief, aliquots of serial dilutions were spread onto plates with PCA (plate count agar, Biokar) and DRBC (Dichloran Rose Bengal Chlorotetracycline agar, Biokar) for psychrotrophic microorganism and yeasts and moulds enumeration, respectively, and placed by inclusion in MRS agar (Man-Rogosa-Sharpe, Biokar) for lactic acid bacteria count. PCA plates were incubated at 6.5 ± 1 °C for 10 days, DRBC plates were incubated at 25 ± 1 °C for 5 days, and MRS plates were incubated at 37 ± 1 °C for 7 days. The results were reported as log Colony Forming Units (CFU) per gram of fresh weight. Three determinations per treatment (three trays) were performed in duplicate at each sampling point.

2.3.3 Consumer acceptability

The consumer acceptability test was conducted under controlled conditions (illumination and temperature) with 16 volunteers from the staff of the research centre. The samples were evaluated as described previously by Altisent et al. (2014). The overall acceptability was expressed as the percentage of consumers satisfied (scoring 6 or more in a 9-point hedonic scale), the percentage of consumers who rated that sample as neither liked nor disliked (score=5), and finally, the percentage of consumers that disliked the product (scoring less than 5 in a 9-point hedonic scale).

2.4 Statistical analysis

All data were evaluated using analysis of variance (ANOVA) with JMP®8 statistical software (SAS Institute, Cary, NC, USA). Significant differences between treatments were analysed by Tukey's Honest Significant Difference (HSD) test at a significance level of $P < 0.05$.

3 Results and discussion

3.1 Selection of most suitable pear cultivar

The firmness values of whole pears were determined before processing and displayed ranges of 37.2-53.9 N, 58.8-67.6 N, 49.0-65.6 N and 41.2-52.9 N for 'Flor de invierno', 'Passe-Crassane', 'Ercolini' and 'Conference', respectively (data not shown). Table 1 presents the physicochemical characteristics of the four studied pear cultivars after processing. Significant differences in soluble solids content (SSC) were observed among cultivars. 'Conference' had the highest SSC value (15.0 %), and 'Ercolini' had the lowest (11.3 %). The titratable acidity (TA) ranged from 1.4 and 1.5 g malic acid L^{-1} ('Conference' and 'Ercolini', respectively) to 2.7 g malic acid L^{-1} ('Passe-Crassane'). After dipping pear wedges in NatureSeal® AS1 (Agricoat), unremarkable changes were observed in SSC and TA (data not shown). The hue angle (h°)

is an indicator to determine the colour of the flesh. The 'Conference' pears presented a more yellowish colour of the flesh (h° 101.4) than 'Ercolini' pears (h° 104.2) attributed to cultivar differences. After 7 days of storage at 5 °C, samples were analysed again. Untreated (water) and NatureSeal® AS1 (NS) pear wedges did not show significant changes in SSC and TA throughout storage (data not shown). Only untreated 'Conference' pear slices experienced a large decrease in flesh firmness (from 15.61 N to 11.04 N) after storage at 5 °C for 7 days, whereas 'Flor de invierno', 'Passe-Crassane' and 'Ercolini' did not show significant declines in firmness (data not shown). When pear slices were treated with NS, no significant differences in firmness were observed after storage for all cultivars (data not shown).

To evaluate the susceptibility of pears to browning during shelf life, the browning index (BI) was evaluated. Browning is one of the majors concerns to fresh-cut processors because it has a direct effect on the consumer's purchase decision. The browning index after processing was significantly different among the pear cultivars ('Flor de invierno': 8.89, 'Passe-Crassane': 14.23, 'Ercolini': 11.70, and 'Conference': 15.70). It is because each cultivar has a different phenolic concentration which is the polyphenol oxidase (PPO) substrate. The increase in the BI after 7 days of storage compared with the initial values is presented in Table 2. The increase in the BI was higher in untreated pears. In treated pear wedges, 'Flor de invierno' was the cultivar that presented the highest increase in BI (6.51). The 'Flor de invierno' pear would not be an appropriate cultivar to be processed because presented the highest ΔBI even with antioxidant treatment. In contrast, ΔBI in 'Ercolini' was the lowest, and no effect of antioxidant treatment was observed.

After 7 days of storage, the headspace gas composition of the packages was measured. In both samples (CK and NS), a strong decrease in O_2 levels was observed, whereas CO_2 levels increased regardless of cultivar. Untreated pear wedges of 'Flor de invierno', 'Passe-Crassane', 'Ercolini' and 'Conference' reached O_2 values of 9.9, 6.1, 0.8 and 5.6 % and CO_2 levels of 8.4, 11.8, 14.2 and 10.8 %, respectively. No significant differences in O_2 and CO_2 levels were observed between untreated and treated pear wedges from 'Passe-Crassane', 'Ercolini' and 'Conference' (data not shown). Nevertheless, in 'Flor de invierno', a slight difference of O_2 level was observed between treated and untreated pear wedges after 7 days. Treated wedges showed lower O_2 levels (1.7 %), whereas untreated wedges did not (9.9 %).

Visual evaluation of the samples after 7 days of storage at 5 °C was conducted. Untreated samples were all below the limit of marketability, but samples treated with NS solution presented excellent visual quality for all tested cultivars (Fig. 1), with 'Conference' and 'Ercolini' obtaining the highest score (between very good and excellent).

After evaluation of different pear cultivars, the 'Conference' pear was selected as the best cultivar. This selection was based on physicochemical characteristics, (high levels of soluble solids content and low acidity) and a low increase in BI. 'Conference' also received the best visual acceptance score after 7 days of storage at 5 °C. Although results were also promising for 'Ercolini', the 'Conference' pear can be stored at low temperature in a controlled atmosphere for a long period of time [22]. This property increases the availability of this cultivar throughout the year, and as a result, a fresh-cut pear product could be produced along the all year compared to 'Ercolini'. In addition in 2014, 198277 tons of pears were

produced in our area (Catalonia), 'Conference' held the first positions in the pear production (87167 tons) while 'Ercolini' was appeared in the fifth position (6960 tons) [1]. Similarly, Arias et al. [3] found that 'Conference' was the most appropriate cultivar among the three studied varieties ('Conference', 'Williams' and 'Passa-Crassane'). This author observed that 'Conference' was the cultivar best suited for minimal processing.

3.2 Selection of the antioxidant treatment

After processing (0 day), the SSC of 'Conference' pears treated with different antioxidants ranged from 13.9 to 15.0 % (Table 3). At the end of evaluation, no significant differences were noted among the SSCs of different treatments (data not shown). For titratable acidity after processing, pears treated with different antioxidants ranged from 1.2 to 1.9 g malic acid L^{-1} (Table 3), and after 14 days , they reached values from 0.9 to 1.2 g malic acid L^{-1} regardless of the treatment applied (data not shown). For the hue angle values, slight differences among treatments were observed initially, and only pear wedges treated with NS were significantly different from the control sample after processing (Table 3). After 14 days of storage at 5 °C, these differences were more significant. When pear wedges were treated with water (CK) or AsAc, the hue angle reached values of 96.6 and 96.0, respectively. Samples treated with CaAs or NaAs had values of 101.3 and 101.5, whereas those samples treated with NS showed the highest value (103.4) (Table 3).

After processing, wedges dipped in different antioxidants reached BI values of CK, 15.70; NS, 12.36; AsAc, 15.47; CaAs, 13.26; and NaAs, 14.00; although only significant differences were observed between samples dipped in water (CK) and NS solution (data not shown). Conversely, after processing (0 day), no significant differences were observed in texture due to the different antioxidants tested (Table 4). Nevertheless, after 14 days, the sample without antioxidant (CK) showed a strong reduction in firmness (from 15.61 to 11.89 N), but firmness was maintained in the remaining samples (14.31 to 16.52 N). After 14 days, the increase in the browning index was higher in untreated and AsAc treated pears (7.50 and 9.01, respectively) than in the other treatments. Treatments that avoided the browning effect in fresh-cut pear surface were NS, CaAs and NaAs which showed browning indexes of 2.58, 1.88 and 3.78, respectively (Table 4).

For the O_2 and CO_2 composition in the headspace, levels of O_2 decreased drastically to 0 % after 7 days regardless of the antioxidant treatment (data not shown). The CO_2 levels increased gradually during storage. AsAc-treated pears had the highest value (29.2 %), and CO_2 values ranged from 24.8 to 25.1 % in untreated and NS- and CaAs-treated pears.

Samples treated with NS solution presented an excellent visual quality (Fig. 2), whereas those samples treated with CaAs and NaAs presented scores near the limit of marketability (good and very good). Untreated and AsAc treated pear wedges received scores below limit of usability (=1).

To minimize visual deterioration of fresh-cut pears, certain reducing agents such as ascorbic acid, 4-hexylresorcinol, cysteine, N-acetylcysteine and sodium eritorbate combined with calcium salts such as calcium chloride, calcium lactate have been investigated [3, 4, 8-11]. The AsAc treatment composed of 20 g L^{-1} ascorbic acid, 10 g L^{-1} citric acid and 10 g L^{-1} calcium chloride obtained the worst results in our study, which is consistent with the results obtained by Arias et al.

(2008). Larrigaudiere et al. [23] studied the effects of chemical preservatives on the oxidative behaviour of fresh-cut 'Fuji' apples and determined the H_2O_2 levels, which are used as a marker for oxidative stress. Ascorbic acid is generally used as antioxidant to prevent oxidation-related processes and to limit the accumulation of H_2O_2. An increase in H_2O_2 levels was observed in fresh-cut pears treated with ascorbic acid. These results might occur because at higher concentration, ascorbic acid might act as a pro-oxidant and therefore tend to have the opposite effect with respect to H_2O_2 accumulation [23, 24], and this is likely what occurred in our treatment. As an alternative anti-browning treatment, other ascorbic salts were evaluated in this study in combination with the most frequently used calcium salt (calcium chloride, $CaCl_2$), which avoided losses in texture. However, these ascorbic salts have not been evaluated previously in fresh-cut pears. We concluded that the use of 20 g L^{-1} calcium ascorbate plus 10 g L^{-1} calcium chloride (CaAs treatment) and 20 g L^{-1} sodium ascorbate plus 10 g L^{-1} calcium chloride (NaAs treatment) as dipping solutions after cutting delivered colour and texture stability and good visual aspects for fresh-cut 'Conference' pears for 14 days of storage at 5 °C. The results confirmed the ability of NS to maintain the freshness of fresh-cut 'Conference' pears, although similar results were obtained with CaAs and NaAs solutions. A similar evaluation of firmness was obtained in samples treated with CaAs and NaAs. However, CaAs was selected for further studies because certain judges found a 'salty' flavour in NaAs-treated pear wedges (data not shown).

3.3 Semi-commercial evaluation

3.3.1 Physicochemical evaluation

Fresh-cut pears before CaAs treatment presented values of 14.3 % for SSC, 1.2 g malic acid L^{-1} for TA and 17.35 N for firmness (Table 5). With respect to flesh colour, wedges showed 103.1 and 13.0 values of hue angle and BI, respectively (data not shown). The results revealed that CaAs application did not modify these physicochemical parameters. During shelf life, no remarkable changes were observed in SSC and TA, but flesh firmness significantly increased after 8 days of storage in both realistic (25.17 N) and cold chain break (27.66 N) storage conditions. This phenomenon was also noted by Xiao et al. [25] in minimally processed 'Anjou' pears and could be due to dehydration of the surface pear tissue during storage, which leads to a hardening of the pear wedge that increased the measured resistance and consequently resulted in higher firmness measurements. A gradual increase of the BI was observed on pear wedges with increasing storage time. The highest increase was observed in samples stored 8 days under cold chain break conditions (4.9).Change in the package headspace gas composition during shelf life was also observed. After 3 days of storage, samples showed a reduction of O_2 levels (6.6 % O_2) and a strong increase in CO_2 levels (9.3 % CO_2). Both samples stored at realistic and cold chain break conditions showed decreased O_2 levels and increased CO_2 levels, although samples stored at 8 °C for 5 days showed the most drastic reduction of O_2 levels and increase of CO_2 levels, e.g., levels of 0.0 % O_2 and levels of 21.5 % CO_2 (data not shown).

3.3.2 Nutritional evaluation

Nutritional parameters were affected by the CaAs treatment. Before treatment, pear wedges showed values of ascorbic acid content, total phenolic content and antioxidant activity of 0.01 g ascorbic acid kg^{-1} (Fig. 3), 0.35 g gallic acid kg^{-1} (Fig. 4) and 0.64 mmoles ascorbic acid kg^{-1} (Fig. 5), respectively. A similar initial phenolic content in fresh-cut 'Conference' pear without treatment was noted by Arias et al. [3] (0.30 g phenols kg^{-1}), and higher content was observed in 'Passe-Crassane' (1.20 g phenols kg^{-1}). Gomes et al. [26] observed that browning development on the tissue surface was affected by pH and the phenolic substrate, and thus polyphenol oxidase (PPO) could develop enzymatic browning in fresh-cut 'Passe-Crassane', which was the likely cause of the increased browning index in this variety.

The ascorbic acid content of untreated fresh-cut pears was 0.01 g ascorbic acid kg^{-1} (Fig. 3), and as a consequence of CaAs treatment, this content increased by 43.9 times (0.60 g ascorbic acid kg^{-1}), by 2.4 times for total phenolic content (from 0.35 to 0.85 g gallic acid kg^{-1}) and by 5.3 times for antioxidant activity (from 0.64 to 3.41 mmoles ascorbic acid kg^{-1}) (Fig. 4 and 5). Our results are consistent with others obtained in the 'Conference' pear [27], which reported that the treatment (10 g L^{-1} ascorbic acid plus 5 g L^{-1} calcium chloride) caused an increase of 60 % in the ascorbic acid content after processing of fresh-cut pear (0.05 g kg^{-1}). After sample treatment, the antioxidant activity increased nearly 5 times. This increase could be due to the composition of the treatment chosen, which contains calcium ascorbate with high antioxidant activity. Oms-Oliu et al. [28] optimized an antioxidant treatment for fresh-cut 'Flor de invierno' pears and did not notice an enhancement in antioxidant activity, but their treatment contained no calcium ascorbate.

During fresh-cut pear shelf life, a significant reduction of all nutritional parameters was noted. Gradual reductions of total phenolic content (Fig. 4) were observed during storage, from 0.85 (0 day) to 0.75 g gallic acid kg^{-1} after 8 days at realistic storage conditions. Nevertheless, the lowest content of total phenol was found at cold chain break storage conditions (0.65 g gallic acid kg^{-1}). In addition, a large significant reduction of ascorbic acid content (Fig. 3) and antioxidant activity (Fig. 5) was observed after 3 days of storage at 4 °C, reaching 0.24 g ascorbic acid kg^{-1} and 1.41 mmoles ascorbic acid kg^{-1}, respectively. After 8 days of storage, a weak reduction was observed in ascorbic acid content and antioxidant activity, which was the similar at both storage conditions. Values of ascorbic acid content ranged from 0.07 to 0.09 g ascorbic acid kg^{-1} and those of antioxidant activity ranged from 1.03 to 1.20 mmoles ascorbic acid kg^{-1}. Soliva-Fortuny and Martín-Belloso [27] also observed that ascorbic acid contents decreased to 0.05 g kg^{-1} after 7 days of storage under MAP conditions.

3.3.3 Microbial quality

Microbial quality changes were not observed between untreated and CaAs-treated fresh-cut 'Conference' pears on the processing day (0 day) (Fig. 6). The count of psychrotrophic microorganisms (PM) after processing and after dipping of wedges ranged from 2.7 to 2.8 log CFU g^{-1} on untreated and treated pear wedges, respectively. For yeasts and moulds (YM), the majority of samples showed values below the limit of detection (LD, 1.4 log CFU mL^{-1}). The counts of lactic acid bacteria (LAB) were below the detection limit (< 0.5 log CFU mL^{-1}) on both on untreated and treated pear wedges.

316 Oms-Oliu et al. [6] and Soliva-Fortuny and Martín-Belloso [27] highlighted the importance of evaluating the
317 microbial stability of minimally processed pears and observed that the main native microbiota of 'Conference' fresh-cut
318 pears stored at 4 °C were moulds and yeasts, but MAP inhibited growth of moulds and yeasts, whereas mesophilic
319 bacteria proliferated rapidly.

320 After 3 days of storage at 4 °C, PM increased to 4.3 log CFU g^{-1}, whereas yeasts and moulds counts were maintained
321 close to the limit of detection (LOD). LAB counts rise above the LOD although no significant differences were observed
322 compared with the initial count. At the final sampling point (8 day), no differences among storage conditions (realistic
323 and cold chain break storage conditions) were observed in YM and LAB. YM and LAB enumeration ranged from 1.6 to
324 2.0 log CFU g^{-1} and from 0.8 to 1.2 log CFU g^{-1}, respectively.

325 Oms-Oliu et al. [6] and Soliva-Fortuny and Martín-Belloso [27] observed that the main microbiota on fresh-cut pear
326 consist of moulds and yeasts, but these could be inhibited because they are sensitive to CO_2. Under MAP storage of fresh-
327 cut pear, CO_2 levels increased during storage and inhibited the proliferation of moulds and yeasts throughout storage, thus
328 facilitating the colonization by populations of bacteria, which were minority microorganisms before processing. These
329 reports support our findings that the moulds and yeasts load was constant during storage, whereas that of psychrotrophic
330 bacteria increased up 5 log CFU g^{-1}. The proliferation of microorganisms on the surface of fresh-cut fruit is currently
331 retarded or inhibited by the use of low storage temperature, modified atmosphere packaging, and antimicrobial substances
332 [29]. With respect to temperature, we noted that under cold chain beak storage conditions, psychrotrophic bacteria
333 showed a weak increase (4.9 log CFU g^{-1}) compared with storage at realistic conditions (4.4 log CFU g^{-1}), although these
334 values were not significantly different.

335 **3.3.4 Consumer assessment: visual quality and consumer acceptability**

336 Immediately after processing, the samples obtained the highest score (excellent) for visual quality, and after 3 days of
337 storage, acceptance was reduced to very good (Fig. 7). After 8 days, when samples were stored at constant temperature (4
338 °C), they received the lowest acceptance (below limit of marketability), whereas samples stored for 3 days at 4 °C plus 5
339 days at 8 °C reached an acceptance score between good and very good.

340 After processing, the consumer acceptability was measured, 92 % of consumers indicated their satisfaction with the
341 fresh-cut pear (Fig. 8). This acceptance increased up to 100 % after 3 days of storage. After 8 days, under realistic cold
342 chain conditions and cold chain break conditions, 44 % of consumers liked the pears. However, fresh-cut pears stored
343 under cold chain break conditions received a greater percentage of unsatisfied consumers (44 %) than those maintained at
344 4 °C over the entire shelf life (25 %).

345 # 4 Conclusions

346 In the current study, a minimally processed pear product was optimized using the 'Conference' pear as the fruit
347 cultivar and treatment with a solution consisting of 20 g L^{-1} (w/v) calcium ascorbate and 10 g L^{-1} (w/v) calcium chloride

solution. The selected treatment was able to minimize visual deterioration after 8 days of storage at 4 °C and under cold chain break conditions. When our selected treatment was applied, increases in the ascorbic acid content, total phenolic content and antioxidant activity of minimally processed pear samples were observed. These values were reduced during shelf life, but the total phenolic content at the final sampling point was greater than that in samples after processing (without treatment). The microbial stability of our fresh-cut pear had the same tendency as that of the other minimally processed pear products evaluated. The total mesophilic aerobic population exhibited faster growth than yeasts and moulds, which did not increase over the shelf life. Our fresh-cut 'Conference' pear product could offer added value to pear production in our area and introduce to the market a product with higher convenience for consumers. For this product, no more than 8 days of shelf life are recommended to ensure consumer satisfaction.

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Statement of Competing Interests

The authors have no competing interests.

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448 **Table 1. Physicochemical parameters of fresh-cut pear cultivars after processing.**

	SSC (%)	TA (g malic acid L^{-1})	Hue angle (h°)
'Flor de invierno'	11.5 ± 0.0 c	2.5 ± 0.1 b	103.0 ± 2.2 ab
'Passe-Crassane'	13.8 ± 0.1 b	2.7 ± 0.0 a	102.3 ± 2.2 ab
'Ercolini'	11.3 ± 0.0 d	1.5 ± 0.1 c	104.2 ± 1.6 a
'Conference'	15.0 ± 0.1 a	1.4 ± 0.0 c	101.4 ± 1.9 b

449

450 Values are expressed as the mean of three values ± standard deviation for SSC and TA and the mean of ten values ±
451 standard deviation for the hue angle. For each parameter, different lowercase letters (a, b, c and d) in the same column
452 indicate significant differences ($p < 0.05$) among pear cultivars according to Tukey's test.

453

454 **Table 2. Variation of the browning index of pear wedges untreated and treated with NatureSeal® AS1 after 7 days of storage at 5 °C.**

ΔBI		
	Untreated wedges	Treated wedges
'Flor de invierno'	11.4 a *	6.5 a
'Passe-Crassane'	13.4 a *	1.8 b
'Ercolini'	3.0 b	2.0 b
'Conference'	10.8 a *	2.5 b

455

456 Values are the mean of thirty values ± standard deviation. Different letters in untreated and treated samples indicate
457 significant differences among cultivars. An asterisk between the untreated and treated columns for each cultivar indicates
458 that significant differences were observed among untreated and treated samples after 7 days of storage according to
459 Tukey's test ($p < 0.05$).

460

461 **Table 3. Physicochemical parameters of fresh-cut 'Conference' pears dipped in different antioxidant solutions.**

Treatment	Initial			After storage (5 °C)	
	SSC (%)	TA (g malic acid L^{-1})	Hue angle (h°)	Hue angle (h°)	
				7 days	14 days
CK	15.0 ± 0.1 a	1.4 ± 0.0 b	101.4 ± 1.8 b	96.5 ± 2.6 d*	96.6 ± 3.2 c*
NS	14.5 ± 0.1 b	1.2 ± 0.0 c	103.7 ± 0.7 a	103.2 ± 1.8 a	103.4 ± 1.5 a
AsAc	14.4 ± 0.1 b	1.9 ± 0.0 a	101.0 ± 1.6 b	98.2 ± 3.1 c*	96.0 ± 2.8 c*
CaAs	13.9 ± 0.0 c	1.2 ± 0.0 c	102.0 ± 2.1 ab	101.3 ± 1.6 b*	101.3 ± 1.9 b
NaAs	14.4 ± 0.0 b	1.2 ± 0.0 c	103.0 ± 1.9 ab	101.7 ± 1.9 ab	101.5 ± 2.1 b

462

463 Values are the mean of three values ± standard deviation for SSC and TA; and the mean of thirty values ± standard
464 deviation for the hue angle. Different letters for the same parameter indicate significant differences among treatments ($p <$
465 0.05) according to Tukey's test. CK: distilled water; NS: 50 g L^{-1} NatureSeal® AS1; AsAc: 20 g L^{-1} ascorbic acid, 10 g L^{-1}
466 citric acid and 10 g L^{-1} calcium chloride; CaAs: 20 g L^{-1} calcium ascorbate and 10 g L^{-1} calcium chloride; NaAs: 20 g L^{-1}
467 sodium ascorbate and 10 g L^{-1} calcium chloride. An asterisk in the hue angle data at 7 and 14 days of storage means that
468 significant differences were observed with respect to the initial value in each treatment.

469

470

471

472

Table 4. Evolution of physicochemical parameters of fresh-cut 'Conference' pears dipped in different antioxidant solutions.

	Storage time	CK	NS	AcAs	CaAs	NaAs
Texture (N)	0 day	15.61 ± 1.48 a A	16.87 ± 3.56 a A	15.97 ± 1.72 a A	15.05 ± 0.97 ab A	17.27 ± 2.65 a A
	7 days	11.04 ± 1.21 b B	12.59 ± 2.20 b AB	13.51 ± 1.59 a A	13.86 ± 0.90 b A	13.67 ± 2.93 b A
	14 days	11.89 ± 1.79 b B	14.31 ± 2.02 ab AB	14.83 ± 1.92 a A	15.74 ± 1.47 a A	16.52 ± 1.84 ab A
Δ BI	7 days	4.02 ± 4.74 A	1.59 ± 3.40 A	2.42 ± 5.23 A	2.37 ± 3.19 A	1.91 ± 3.92 A
	14 days	7.50 ± 6.33 A	1.68 ± 2.79 C	6.80 ± 4.91 AB	3.35 ± 4.28 C	3.78 ± 4.52 BC

Values are the mean of nine values ± standard deviation for texture. Values are the mean of thirty values ± standard deviation for ΔBI. For each parameter, different lowercase letters (a, b and c) in the same column indicate significant differences ($p < 0.05$) among sampling days according to Tukey's test. Different uppercase letters (A, B, C, D and D) in the same row indicate significant differences ($p < 0.05$) among treatments. CK: distilled water; NS: 50 g L^{-1} NatureSeal® AS1; AsAc: 20 g L^{-1} ascorbic acid, 10 g L^{-1} citric acid and 10 g L^{-1} calcium chloride; CaAs: 20 g L^{-1} calcium ascorbate and 10 g L^{-1} calcium chloride; NaAs: 20 g L^{-1} sodium ascorbate and 10 g L^{-1} calcium chloride.

Table 5. Physicochemical parameters of fresh-cut 'Conference' pears during semi-commercial assay.

Sampling time	SSC (%)	TA (g malic acid L^{-1})	Texture (N)	ΔBI
before CaAs treatment	14.3 ± 0.1 a	1.2 ± 0.1 ab	17.4 ± 0.5 b	
after treatment (0 day)	14.4 ± 0.0 a	1.3 ± 0.0 a	19.9 ± 2.1 b	1.8 ± 2.0 b
3 days (4 °C)	13.3 ± 0.3 b	1.1 ± 0.0 ab	16.4 ± 1.5 b	2.8 ± 2.6 b
8 days (4 °C)	13.9 ± 0.2 ab	1.0 ± 0.2 ab	25.2 ± 6.6 a	3.6 ±3.6 ab
8 days (3 d 4 °C + 5 d 8 °C)	13.9 ± 0.3 a	0.9 ± 0.1 b	27.7 ± 3.7 a	4.9 ± 2.6 a

Values are the mean of three values ± standard deviation for SSC and TA; the mean of nine values ± standard deviation for firmness; and the mean of ten values ± standard deviation for ΔBI. Different letters in the same parameter indicate significant differences among samples during shelf life ($p < 0.05$) according to Tukey's test.

Figure caption

Fig. 1. Overall visual quality of wedges from four pear cultivars after 7 days at 5 °C treated with antioxidant solution (NS; 50 g L^{-1} NatureSeal® AS1) or without treatment (CK; water). The data presented are the means of the visual evaluations of three trays per treatment and cultivar, and bars represent the standard deviation of the mean.

Fig. 2. Overall visual quality of fresh-cut 'Conference' pear after 7 and 14 days at 5 °C treated with different antioxidant solutions (CK: distilled water; NS: 50 g L^{-1} NatureSeal® AS1; AsAc: 20 g L^{-1} (w/v) ascorbic acid, 10 g L^{-1} (w/v) citric acid and 10 g L^{-1} (w/v) calcium chloride solution; CaAs: 20 g L^{-1} (w/v) calcium ascorbate and 10 g L^{-1} (w/v) calcium chloride solution; NaAs: 20 g L^{-1} (w/v) sodium ascorbate and 10 g L^{-1} (w/v) calcium chloride solution). The data presented are the means of the visual evaluations of three trays per treatment, and bars represent the standard deviation of the mean.

Fig. 3. Ascorbic acid content of fresh-cut pears during shelf life at realistic (4 °C) and simulated cold chain break conditions (g per kg of fresh weight). The data presented are the means of three values. Different letters indicate significant differences ($p < 0.05$). Vertical bars represent the standard deviation of the means.

Fig. 4. Total phenolic content of fresh-cut pear during shelf life at realistic (4 °C) and simulated cold chain break conditions (g gallic acid per kg of fresh weight). The data presented are the means of three values. Different letters indicate significant differences ($p < 0.05$). Vertical bars represent the standard deviation of the means.

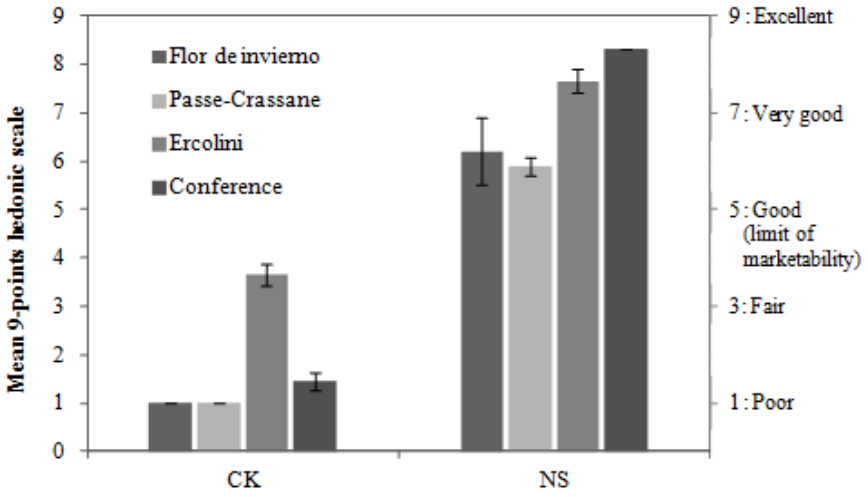
Fig. 5. Antioxidant activity of fresh-cut pears during shelf life at realistic (4 °C) and simulated cold chain break conditions (mmoles ascorbic acid equivalent per kg of fresh weight). The data presented are the means of three values. Different letters indicate significant differences ($p < 0.05$). Vertical bars represent the standard deviation of the means.

Fig. 6. Population of psychrotrophic microorganisms (PM), yeasts and moulds (YM), and lactic acid bacteria (LAB) (log CFU g^{-1}) in fresh-cut 'Conference' pears during shelf life at realistic (4 °C) and simulated cold chain break conditions. Data represent the mean of three determinations, and bars represent the standard deviation of the mean. Different letters indicate significant differences among days ($p < 0.05$).

Fig. 7. Overall visual quality of fresh-cut 'Conference' pears during shelf life at realistic (4 °C) and simulated cold chain break conditions. The data presented are the means of the visual evaluations of three trays at each sampling time, and bars represent the standard deviation of the mean.

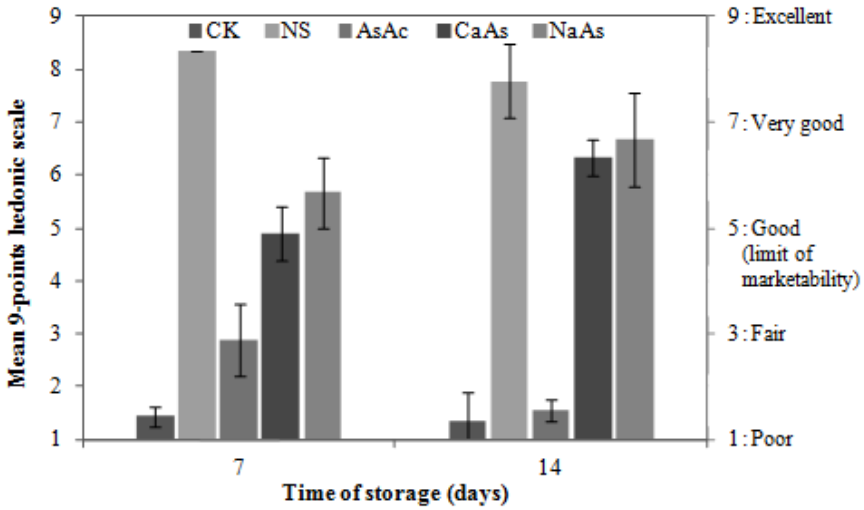
Fig. 8. Percentage of consumers that liked, neither liked nor disliked, and disliked the fresh-cut pear during the shelf life according to overall acceptance.

Fig. 1


Mean 9-points hedonic scale
9
8
7
6
5
4
3
2
1
0
Flor de invierno
Passe-Crassane
Ercolini
Conference
CK
NS
9 : Excellent
7 : Very good
5 : Good (limit of marketability)
3 : Fair
1 : Poor

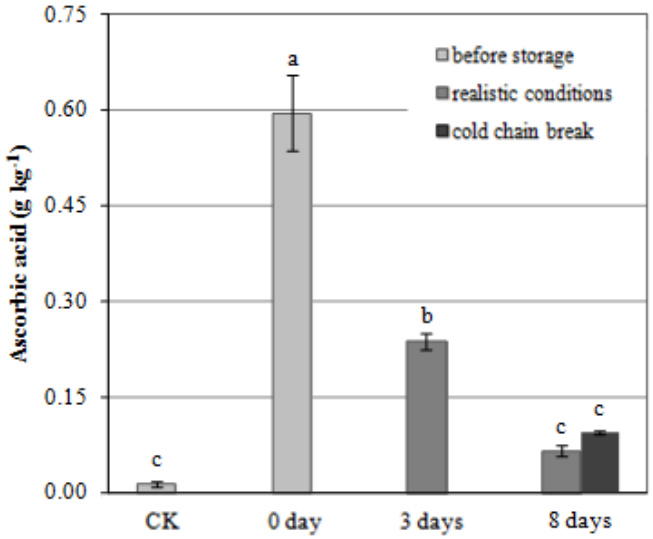
539

Fig. 2


CK
NS
AsAc
CaAs
NaAs
Mean 9-points hedonic scale
9
8
7
6
5
4
3
2
1
9 : Excellent
7 : Very good
5 : Good (limit of marketability)
3 : Fair
1 : Poor
7
14
Time of storage (days)

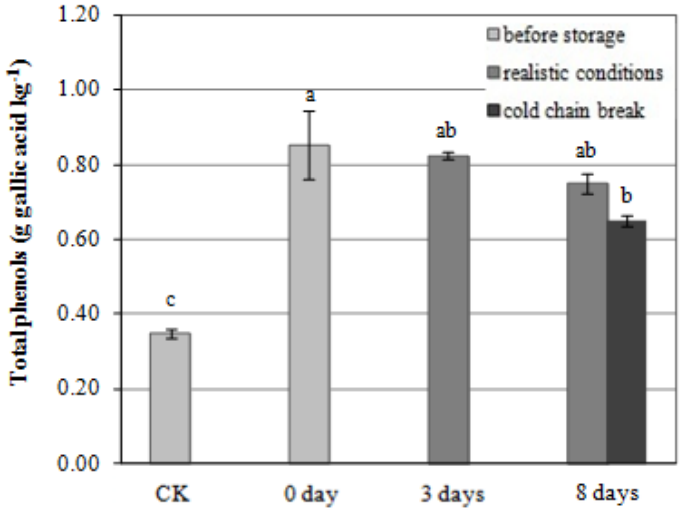
540

Fig. 3


0.75
0.60
0.45
0.30
0.15
0.00
Ascorbic acid (g kg-1)
before storage
realistic conditions
cold chain break
a
b
c
c
c
CK
0 day
3 days
8 days

541

Fig. 4


Totalphenols (g gallic acid kg-1)
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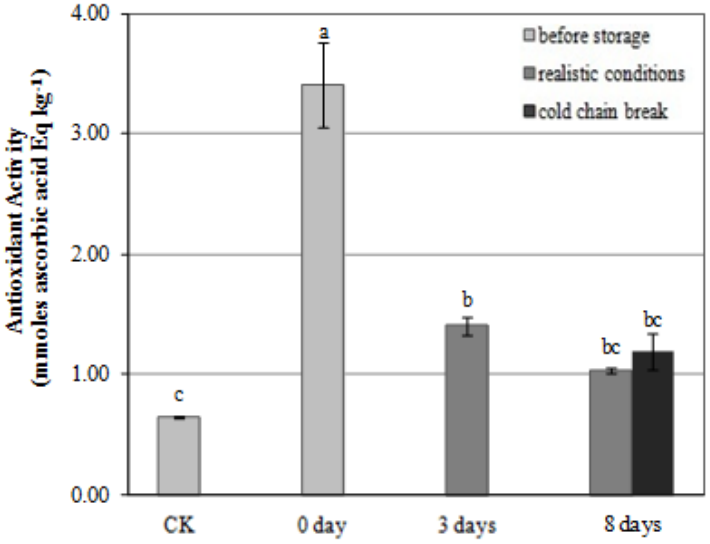
1.00
0.80
0.60
0.40
0.20
0.00
before storage
realistic conditions
cold chain break
a
ab
ab
b
c
CK
0 day
3 days
8 days

542

Fig. 5


Antioxidant Activity (nmoles ascorbic acid Eq kg-1)
4.00
3.00
2.00
1.00
0.00
before storage
realistic conditions
cold chain break
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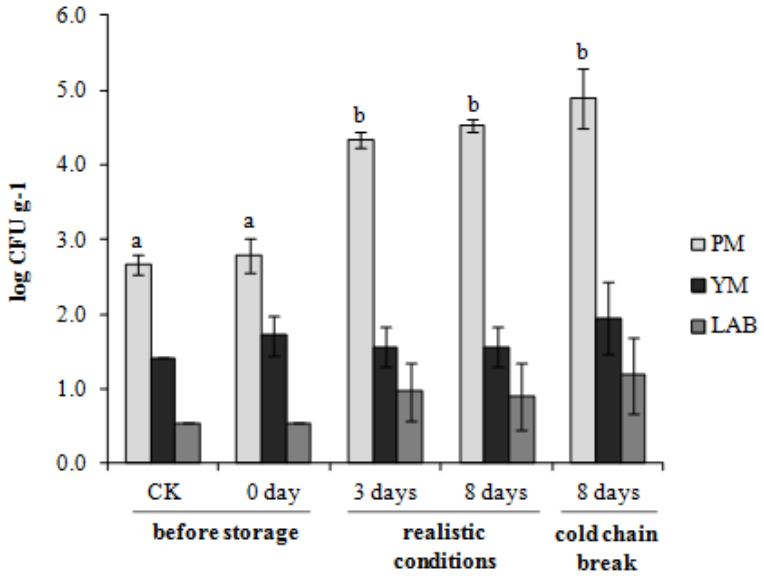
b
bc
bc
c
CK
0 day
3 days
8 days

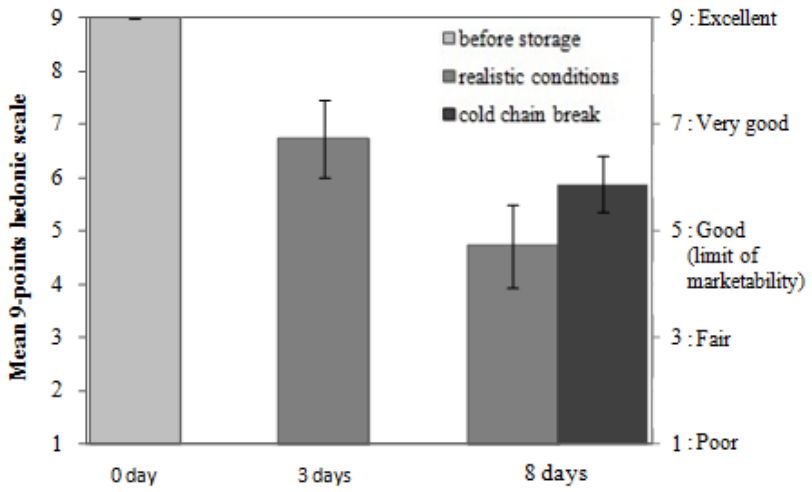
543

Fig. 6


6.0
5.0
4.0
3.0
2.0
1.0
0.0
log CFU g-1
a
a
b
b
b
PM
YM
LAB
CK
0 day
3 days
8 days
8 days
before storage
realistic conditions
cold chain break

544

Fig. 7


Mean 9-points hedonic scale
9
8
7
6
5
4
3
2
1
0 day
3 days
8 days
before storage
realistic conditions
cold chain break
9 : Excellent
7 : Very good
5 : Good (limit of marketability)
3 : Fair
1 : Poor

545

Fig. 8

Overall acceptance (%)			
0 day	Before storage	Like	92
		Neither like or dislike	0
		Dislike	8
3 days	Realistic conditions (4°C)	Like	100
		Neither like or dislike	0
		Dislike	0
8 days	Realistic conditions (4°C)	Like	44
		Neither like or dislike	31
		Dislike	25
	Cold chain break (8°C)	Like	44
		Neither like or dislike	13
		Dislike	44

546