

## REGULAR ARTICLE

# Effect of chitosan coating on quality and nutritional value of fresh-cut 'Rocha' pear

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## Abstract

One of the greatest barriers to the commercial marketing of fresh-cut fruit is limited shelf-life due to tissue softening and surface browning. Moreover, the development of microorganisms on the fruit surface can compromise the safety of the fresh-cut product. Edible coatings have been proposed as postharvest treatments to maintain quality and prolong shelf-life. The present work aims to evaluate the effect of chitosan coatings in physical, chemical, nutritional and microbiological characteristics of minimally processed 'Rocha' pear. Fruits were peeled, cut into slices and immersed in different chitosan solutions (0.7, 1, 1.5 e 2 g L<sup>-1</sup>). Treated samples were stored at 4°C during 10 days. Physical and physicochemical analysis were performed, including moisture content, firmness, color, titratable acidity and total soluble solids. Nutritional analysis and microbiological evaluations of psychrophilic bacteria, molds and yeasts were also carried out. Chitosan coatings reduced water loss, maintained firmness, and reduced surface browning during storage. No negative effects were observed in the nutritional quality of treated pears. Chitosan coatings could be used to preserve quality and to extend shelf-life of fresh-cut 'Rocha' pear.

*Key words:* Biofilms, Edible coatings, Minimally processed fruits, 'Rocha' pear, Shelf life

## Introduction

In the last few years, fresh-cut fruits are increasingly in demand as ready-to-eat products. This increase is mainly due to the importance that the consumers of all ages are giving to the fresh, healthy, and low calorie food products.

The Fresh-cut Produce Association defines a fresh-cut product as any fruit or vegetable that has been physically altered from its original form, but remains in a fresh state ([www.fresh-cuts.org](http://www.fresh-cuts.org)), that it has been trimmed, peeled, washed and cut into 100% usable product that is then bagged or pre-packaged to offer consumers high nutrition, convenience, flavour and value while still maintaining freshness.

Nevertheless, several physical and physiological alterations provoked by wounding

during product preparation accelerate loss of fresh-cut vegetables quality (Saltveit, 1997). Foremost among these, are the removal of the protective epidermal layer of the produce and the exposure of internal cells. These changes not only increase the risk of microbial growth and contamination and facilitate tissues dehydration, but also facilitate several enzymatic reactions, since many enzymes and substrates liberated from the broken cells become mixed (Brecht, 1995; Saltveit, 1997). A means to preserve all the natural and beneficial components of fresh-cut fruits is coating them with an edible material, a coating. Traditionally, edible coatings have been used in the fresh-cut industry as a strategy to reduce the deleterious effects that minimal processing imposes on intact vegetable tissues (Dhall, 2013). Edible coatings act as barriers against moisture loss and gas exchanges, and reduce solute migration, respiration and oxidative reaction rates (Park, 1999). Moreover, coatings reduce the proliferation of spoilage microorganisms on the surface of the fresh-cut produce.

Recently, chitosan has been successfully used as a food wrap because of its film-forming properties (No et al., 2007). Chitosan is a high molecular weight cationic polysaccharide obtained

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from alkaline deacetylation of chitin, a homopolymer of  $\beta$ -(1-4)-N-acetyl-D-glucosamine, which is commercially extracted from shrimp and crab shells. Chitosan coatings are particularly promising because of its biocompatibility, biodegradability, non-toxicity and antimicrobial properties (No et al., 2007; Toan, 2009).

Antimicrobial activity of chitosan has been demonstrated against bacteria, yeasts and some strains of filamentous fungi (Rhoades and Roller 2000). Several models have been proposed to explain this inhibitory activity, being the most probable the interaction between the positive charges of chitosan molecules and the negative charges on microbial cell membrane. Briefly, the electrostatic interaction promotes changes in the membranes permeability, induces hydrolysis of cell wall peptidoglycan and consequently causes growth inhibition or death of microorganisms (Young and Kauss, 1983).

In the last years, chitosan has been used to maintain the quality of several fresh-cut products such as papaya (González-Aguilar et al., 2008), strawberries (Campaniello et al., 2008), mango (Chien et al., 2007), carrots (Simões et al., 2009), among others.

'Rocha' pear (*Pyrus communis* L. cv Rocha) is an exclusive Portuguese variety which production is of relevant economic importance in Portugal (c.a. 195000 tn in 2013, data from Associação Nacional de Produtores de Pêra Rocha), accounting for 95% of the national pear production, mainly concentrated in the west region of the country.

The desirable taste, crisp texture, high digestibility, as well as a high content of phenolic antioxidants (Salta et al., 2010) of 'Rocha' pears make them very attractive and popular among consumers. The main problems that make fresh-cut 'Rocha' pear a perishable product are browning, loss of firmness and microbial growth on the surface.

In this context, the main objective of this study was to evaluate the efficacy of chitosan, as an edible coating, to extend the shelf-life of fresh-cut 'Rocha' pear. The effect of different chitosan concentrations on physicochemical, nutritional and microbiological parameters was evaluated in pear slices stored at 4°C.

## Materials and Methods

### Fruits

'Rocha' pears (*Pyrus communis* L.) were hand-harvested at the stage of commercial maturity and stored in controlled atmospheres, at 1°C and 90-95% relative humidity until use. Fruits were

selected for uniform size and maturity, discarding those with pathological or mechanical injuries and physiological defects, surface-disinfected with a sodium hypochlorite solution (120 ppm Cl<sub>2</sub>), rinsed with tap water and air-dried at room temperature.

### Chitosan coating solutions

Chitosan solutions were prepared by dissolving medium molecular weight chitosan (Sigma-Aldrich), 75-85% deacetylated, in 0.5% ascorbic acid (Fluka), under continuous stirring during 1h. Four chitosan concentrations were used: 0.7, 1, 1.5 and 2 g L<sup>-1</sup>. The pH of the solutions was adjusted to 6.0 with 1N NaOH (Merck).

### Fresh-cut pear treatment

After disinfecting, the pears were manually peeled and cut into slices, approximately 5-6 mm thick, with a sharp stainless steel knife. For each treatment, slices cut from different pears, were dipped for 1 min in the chitosan solution, well-drained at 4°C during 3 min, placed into sterile Petri dishes (3 slices each) and sealed with parafilm. Slices treated with ascorbic acid 0.5% were used as control. Nine trays per treatment were stored at 4°C during 10 days. Assays were performed twice independently.

### Color

The color of the slices was determined with a Minolta chromameter (model CR-300, Data Processor 301, Minolta, USA). Eighteen measurements were evaluated from each treatment, at different time intervals. The color values were expressed by the CIELAB colorimetric system. The L\* value was used as an indicator of loss of brightness. The a\* and b\* values were used to determine chroma (C\*) and hue angle (Ho = 180o + Tang-1 [b\*/a\*]) (Lidon et al., 2012).

### Firmness

The firmness of the pear tissues was measured with a texturometer (Texture Analyser TA-HDI) with a 3 mm diameter flat-head stainless-steel cylindrical probe at a speed of 1,7 mm/s. After 10 days of storage, eighteen measurements were evaluated from each treatment. Data were acquired using Texture Expert software. The firmness was expressed as the maximum force, in newtons (N), required to shear the sample.

### Water content

The water content of the pear slices after 10 days of storage at 4°C was determined by the difference of weight after 72h at 45°C. For each treatment, three replicates of 20 g samples were placed into previously tared Petri dishes and dried.

The results were expressed in percentage according to the formula: water content % =  $(W_i - W_f) / W_i \times 100$ , where  $W_i$  is the initial weight and  $W_f$  is the final weight.

#### **Titrateable acidity and total soluble solids**

Pear samples were mashed and 20 mL of the pulp obtained was homogenized with 20 mL of distilled water. The homogenate was filtered and then titrated with 0.10 N NaOH to pH 8.2. The volume of NaOH needed was used to calculate the titrateable acidity (TA), applying a multiplication factor of 0.67. The results were expressed as g malic acid L<sup>-1</sup> of juice.

The total soluble solids (TSS) content of the fruit pulp was determined by using a hand-held refractometer (Atago ATC-1E) at 20°C. The results were expressed in °Brix (AOAC 2000).

#### **Sugars, phenolic compounds and vitamin C content**

20 grams of pulp were homogenized with 100 mL of deionised water, centrifuged at 15000 g (Biofuge 28 RS) for 15 min at 4°C and extracted following Hudina and Stampar (2000). Sugar separation and quantification was performed using an HPLC system (Waters, USA) equipped with a Refractive Index Detector (2414 Waters), a reverse Sugar-Pak1 Column (300 x 6.5 mm, Waters), at 90°C, with H<sub>2</sub>O as eluent (containing 50 mg/L EDTA-Ca) and a flow rate of 0.5 mL/min. Samples of 30 µL were injected. Sucrose, fructose, glucose and sorbitol standards were used for sugars identification and quantification in the samples.

Phenolic compounds were extracted and measured according to Vieira et al. (2009). Two grams of pulp were homogenized with 20 mL of acetone (80%), then submitted, for 15 min, to an ultrasound bath, and centrifuged (1000 g, 10 min, 5°C). After mixing the supernatant (0.1 mL) with deionized water (2.5 mL), Folin-Ciocalteu reagent (0.5 mL) was added. After 5 min, 1.5 mL of a sodium carbonate solution (at 20%) was added and the final volume completed to 10 mL. Two hours later, the absorbance was measured at 760 nm (Shimadzu UV-160 spectrophotometer), using a standard curve with gallic acid (50, 100, 150, 250 and 500 mg L<sup>-1</sup>). The results were expressed as mg EGA/100 g fresh weight.

For vitamin C determination, samples of 20 g of pulp were homogenized with 30 mL of 6% metaphosphoric acid (Sigma) and centrifuged at 15000 g for 25 min at 4°C (Albuquerque et al., 2005). Filtered samples (20 µL aliquots) were analyzed using a HPLC system (Beckman System Gold)

equipped with a diode array detector (λ = 254 nm) operated by a Gold 8.10 software and an Aminex HPX-87H column (BioRad). The mobile phase was 5 mM H<sub>2</sub>SO<sub>4</sub> (pH 2.3) at room temperature, with a flow rate of 0.4 mL/min. Three replicates of each sample were injected. An internal standard of ascorbic acid (Sigma) was used.

#### **Microbiological counts**

Microbiological counts were determined at 10 days of storage period. Ten grams of sample was mixed with 90 mL 0.1% (w/v) peptone solution and homogenized for 1 min using a blender (Braun minipimer); subsequently, 10-fold dilutions were made in 0.1% (w/v) peptone solution. Psychotrophic bacteria, yeast and mold counts were performed by the pour-plate method. For bacteria count, PCA plates were incubated at 4-5°C during 10 days, and for yeast and mold counts, PDA plates were incubated at 25°C during 72h. Three samples per treatment were used. Microbial counts were reported as log CFU/g fresh weight.

#### **Statistical analysis**

All the results were submitted to analysis of variance (ANOVA). The mean values were compared by using Tukey test. The statistical significance was assessed at P < 0.05.

#### **Results and discussion**

Fresh-cut fruits are more perishable than the corresponding whole uncut produce. Wounding during preparation provokes negative effects on product quality such as browning, off-flavour development, loss of firmness and microbial development on the fruit surface (Kader, 2002). Among these, enzymatic browning is frequently the major limiting factor of the shelf-life in fresh-cut susceptible fruit, such as 'Rocha' pear. In the present study, the effect of chitosan coatings on the quality of fresh-cut 'Rocha' pear was evaluated in slices dipped during 60s in solutions with different chitosan concentrations kept at 4°C during 10 days.

The effect of chitosan coatings on the color of pear slices is showed in Figure 1. The changes of brightness during storage followed a similar trend in all the chitosan treatments applied, although with small differences among them (Figure 1A). However, significant differences were observed between the control slices and those treated with chitosan. After 72h of storage, the control slices started to lose lightness. Chitosan treatments significantly delayed tissue browning, being likewise efficient all the concentrations above 0.7 g L<sup>-1</sup> (Figure 1A).

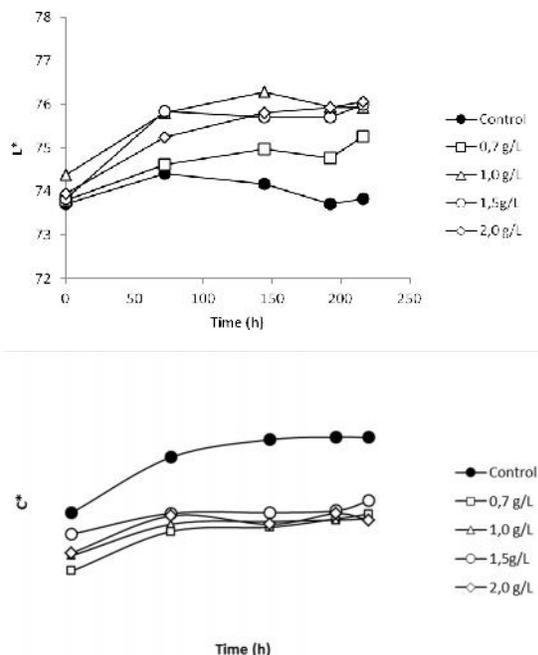


Figure 1. Effect of chitosan treatments on the cut surface color of 'Rocha' pear slices kept at 4°C. Data shown are the means for replicates of 18 slices each. L\*, lightness; C\*, chroma.

Slice browning was also characterized by a decrease in hue angle and an increase in chroma. The decrease in hue angle followed a similar trend in all the treatments, with values diminishing from about 106 to 97 during the storage (data not shown). After 10 days, only a slight difference was observed between control and chitosan treated slices (Table 1). On the other hand, significant differences were observed in chroma between control and treated slices, during all the storage period (Figure 1B). Browning was slower and less intense in pear slices treated with chitosan. However, the effect of the different chitosan concentrations was no significantly different.

A similar effect of chitosan on preserving color was observed in other fresh-cut fruits such as strawberries (Campaniello et al., 2008), papaya (Gonzalez-Aguilar et al., 2008), peach, pear and kiwifruit (Du et al., 1997) and mango (Chien et al., 2007).

Tissue browning following wounding is usually a result of oxidative reactions mediated by polyphenoloxidase (PPO). PPO has a relatively high affinity to oxygen and, after cutting, catalyzes a fast process in which phenolic compounds are oxidized into o-quinones leading

to the formation of undesirable dark brown pigments (Martinez and Whitaker, 1995; Toivonen and Brummell, 2008). Modified atmosphere packaging and refrigeration are not enough to completely avoid tissue browning. Thus, to overcome this limitation, different approaches were studied, such as the use of edible coatings and/or natural antibrowning compounds. Chitosan coatings, per se, were able to decrease browning in fruits by reducing polyphenol oxidase and peroxidase activities (Zhang and Quantick, 1998). This effect was directly related to the modification of the internal atmosphere in the fruit, with decreased levels of O<sub>2</sub> and increased levels of CO<sub>2</sub>.

In order to enhance the antibrowning activity, in this work was tested a combination of a chitosan coating with a natural antibrowning agent. As chitosan is insoluble in aqueous solutions but soluble in weak acid solutions, chitosan was dissolved using a 0.5% ascorbic acid solution. Ascorbic acid is a well-known antioxidant that has been successfully used to reduce enzymatic browning in susceptible pome fruits such as apples (Baldwin et al., 1996; Lee et al., 2003) and pears (Gorny et al., 2002; Krasnova et al., 2013). Ascorbic acid reduces the o-quinones, generated by the action of PPO, back to the phenolic substrates (Rojas-Grau et al., 2009). However, ascorbic acid is oxidized to dehydroascorbic acid after a certain time, allowing the accumulation of o-quinones again.

Since chitosan coating creates a semi-permeable barrier that controls gas exchange, reducing the contact of the exposed fruit surface to oxygen, the combined effect of chitosan coating plus ascorbic acid resulted in an effective way to control browning in 'Rocha' pear slices.

Moreover, the antibrowning capacity of the coating was further enhanced by increasing the pH of the chitosan solutions (final pH adjusted to 6). Taking into account that the maximum activity of pear PPO occurs at pH values between 4.3 and 5.5 (Espín et al., 1998; Siddiq et al., 1994), it was expected that chitosan solutions adjusted to pHs closer to neutral could reduce PPO activity, thus enhancing the antibrowning effect of the coating. In fact, when pear slices were treated with chitosan solutions with acidic pH, around 3, no differences were observed between control and chitosan treated slices (data not shown). Moreover, a significant increase in browning and softening was observed in all the treated samples.

Table 1. Effect of chitosan treatments on Hue angle, firmness, water content, total soluble solids (TSS), pH and titratable acidity (TA) of 'Rocha' pear slices after 10 days storage at 4°C.

Parameter	Chitosan (g L <sup>-1</sup> )				
	0.0	0.7	1.0	1.5	2.0
Hue <sup>o</sup>	97.20 ± 1.98 <sup>a</sup>	98.70 ± 1.45 <sup>a</sup>	98.50 ± 2.15 <sup>a</sup>	98.70 ± 1.25 <sup>a</sup>	98.90 ± 1.89 <sup>a</sup>
Firmness (N)	7.65 ± 0.23 <sup>a</sup>	7.22 ± 0.43 <sup>a</sup>	7.40 ± 0.98 <sup>a</sup>	10.52 ± 0.70 <sup>b</sup>	8.68 ± 0.96 <sup>a</sup>
Water content (%)	85.70 ± 3.19 <sup>a</sup>	86.66 ± 2.08 <sup>a</sup>	86.91 ± 1.54 <sup>a</sup>	86.87 ± 1.98 <sup>a</sup>	87.10 ± 2.50 <sup>a</sup>
TSS (°Brix)	11.50 ± 0.14 <sup>a</sup>	11.60 ± 0.28 <sup>a</sup>	11.20 ± 0.28 <sup>a</sup>	11.40 ± 0.85 <sup>a</sup>	11.50 ± 0.71 <sup>a</sup>
pH	4.79 ± 0.01 <sup>a</sup>	4.81 ± 0.01 <sup>a</sup>	4.74 ± 0.03 <sup>a</sup>	4.73 ± 0.04 <sup>a</sup>	4.74 ± 0.01 <sup>a</sup>
TA (g malic acid L <sup>-1</sup> )	1.62 ± 0.30 <sup>a</sup>	1.65 ± 0.23 <sup>a</sup>	1.97 ± 0.28 <sup>b</sup>	1.91 ± 0.02 <sup>b</sup>	1.69 ± 0.05 <sup>a</sup>

Mean values ( $n = 18$  for Hue angle and firmness, and  $n = 3$  for other parameters) followed by the same letter are not significantly different ( $p > 0.05$ )

Table 2. Contents of total phenols, ascorbic acid and sugars of pear slices (control and treatments) after 10 days at 4°C. All values are shown as mean value ( $n=3$ ). Different letters indicate significant differences ( $p > 0.05$ ) among treatments.

Chitosan (g L <sup>-1</sup> )	Total Phenols (mg 100 g <sup>-1</sup> )	Ascorbic acid (mg 100 g <sup>-1</sup> )	Sugars (g 100 g <sup>-1</sup> )			
			Sucrose	Glucose	Fructose	Sorbitol
0.0	62.9±11.6 <sup>a</sup>	1.36±0.09 <sup>a</sup>	0.55±0.16 <sup>a</sup>	0.97±0.15 <sup>a</sup>	4.20±0.59 <sup>a</sup>	1.39±0.26 <sup>a</sup>
0.7	57.5±15.3 <sup>a</sup>	1.30±0.06 <sup>a</sup>	0.51±0.01 <sup>a</sup>	1.07±0.11 <sup>a</sup>	4.59±0.65 <sup>a</sup>	1.55±0.13 <sup>a,b</sup>
1.0	60.2±10.3 <sup>a</sup>	0.89±0.10 <sup>b</sup>	0.55±0.19 <sup>a</sup>	1.04±0.10 <sup>a</sup>	4.58±0.73 <sup>a</sup>	1.58±0.06 <sup>a,b</sup>
1.5	59.3±15.5 <sup>a</sup>	0.42±0.04 <sup>c</sup>	0.63±0.08 <sup>a</sup>	1.14±0.12 <sup>a</sup>	4.85±0.55 <sup>a</sup>	1.76±0.01 <sup>b</sup>
2.0	79.8±15.7 <sup>b</sup>	0.54±0.03 <sup>c</sup>	0.63±0.01 <sup>a</sup>	1.08±0.20 <sup>a</sup>	4.45±0.83 <sup>a</sup>	1.67±0.27 <sup>a,b</sup>

All the values are means of three replications + SD. Values within a column followed by the same letter are not significantly different ( $p > 0.05$ )

Table 3. Effect of chitosan treatments on psychrophilic bacteria, yeast and mold counts in 'Rocha' pear slices after 10 days of storage at 4°C.

Microorganism (log CFU g <sup>-1</sup> )	Chitosan (g L <sup>-1</sup> )				
	0.0	0.7	1.0	1.5	2.0
psychrophilic bacteria	6.6 <sup>a</sup>	6.4 <sup>a</sup>	6.0 <sup>a, b</sup>	5.8 <sup>b</sup>	5.7 <sup>b</sup>
yeast and mold	2.6 <sup>a</sup>	2.3 <sup>a</sup>	3.2 <sup>a</sup>	3.0 <sup>a</sup>	ND

Mean values ( $n = 3$ ) followed by the same letter are not significantly different ( $p > 0.05$ ). ND, not detected.

A similar effect of pH was previously observed by Gomes et al. (2010), 'Rocha' pear slices had less browning when treated with neutral solutions than with acidic solutions.

The desirable crisp texture of 'Rocha' pears is one of the attributes that differentiate this variety and make it very attractive and popular among consumers. Thus, the loss of firmness is an important factor to determine the quality and the shelf life of fresh-cut pear. The effect of chitosan coatings on texture is presented in Table 1. After 10 days at 4°C, slices coated with the higher chitosan concentrations presented the higher firmness values. The chitosan concentration of 1.5 g L<sup>-1</sup> was the most efficient to preserve the texture quality, maintaining the original firmness of fresh-cut pear up to day 10.

Similar results were previously reported for fresh products coated with chitosan, such as tomatoes (El Ghaouth et al., 1992), strawberries (Hernandez-Muñoz et al., 2008), papaya

(Gonzalez-Aguilar et al., 2008), mangoes (Nongtaodum and Jangchud, 2009), among others.

The changes observed in the texture of fresh-cut produces may result, mainly, from water loss or from the action of pectinolytic and proteolytic enzymes, which are released due to cell wall rupture after cutting. In our case, even though the water loss was lower as higher the concentration of chitosan used, no significant differences were observed between the control and the treated slices at the end of the storage period (Table 1). The loss of weight during storage was very similar for all the treatments. These results suggested that the effect of chitosan on 'Rocha' pear slices firmness is more related to enzymatic inhibition than to water loss. In fact, it has been previously described that chitosan is capable of inactivating or inhibiting several enzymes that cause deterioration in fruits and vegetables (Bhaskar-Reddy et al., 2000; Bautista-Baños et al., 2006; Gonzalez-Aguilar et al., 2008). These authors

related a significant reduction in activity of enzymes such as polygalacturonase, pectin methylesterase, -galactosidase and proteinases.

During the storage, a small increase of pH (1.5%) and a decrease of about 10% in TSS were observed for all the treatments (data not shown). However, after 10 days of storage, no significant differences ( $p < 0.005$ ) were observed between the control and the treated slices (Table 1); these parameters seemed to be not influenced by chitosan treatments.

After cutting, the increase of respiration rate may be reflected in the changes in titratable acidity of fresh-cut pear (Olivas et al., 2003; King et al., 2012). In fact, during the storage, the control slices showed a decrease in TA of about 45% (from 3 to 1.62 g malic acid L<sup>-1</sup>). Slices treated with 1 and 1.5 g L<sup>-1</sup> chitosan exhibited the lowest decrease (about 33%), reaching final values of 1.97 and 1.91 g malic acid L<sup>-1</sup>, respectively (Table 1). The same effect of chitosan on TA was previously observed in pears (Xu et al., 2013). The high TA content can be attributed to slower ripening and respiration rates in coated than in uncoated slices. Chitosan coating controls the availability of O<sub>2</sub> and CO<sub>2</sub>, playing a key role in inducing the slower ripening rate of pear samples (Perdones et al., 2012). Furthermore, as organic acids, including citric acid and malic acid, are used as substrates for respiration, a reduction on respiration rate implies higher TA values (Bico et al., 2009).

Concerning nutritional parameters, no significant differences ( $p < 0.005$ ) in sugars were observed between coated and uncoated pear slices after 10 days of storage at 4°C (Table 2), except for sorbitol in samples treated with 1.5 g L<sup>-1</sup> chitosan. In general, chitosan treatments had no negative effect on sugar composition. However, the concentration of all the sugars decreased during the storage period. Sucrose showed the higher reduction (30%), followed by sorbitol (about 27%), fructose (10%) and finally glucose (8.5%).

Regarding vitamin C content, a reduction of at least 50% was observed in all the slices during the storage time. Conversely to what was observed by other authors (Rojas-Grau et al., 2009; Xu et al., 2013), our results showed that after 10 days of storage, the levels of vitamin C in slices treated with chitosan were lower than in the control slices (Table 2). These authors reported a protective effect of chitosan coatings on vitamin C, which was not evident in 'Rocha' pear slices, at least at the concentrations of chitosan studied. On the

other hand, Simões et al (2009) suggested that, in carrots, chitosan promoted vitamin C loss by generating reactive oxygen species, which are scavenged by antioxidant compounds like vitamin C.

In general, no significant differences were observed in the total phenolic content of coated and uncoated pear slices at the end of the storage time (Table 2), except for the higher chitosan concentration. When comparing with the control slices, a significant ( $p < 0.005$ ) increase of about 27% was observed.

Regarding microbial growth, chitosan coatings showed a poor inhibitor effect on the growth of psychrophilic bacteria (Table 3). Even with the higher chitosan concentrations, the final counts in pear slices were elevated. However, coatings with 1.5 and 2 g L<sup>-1</sup> chitosan showed a significant logarithmic reduction when compared with the control slices. These results suggest that chitosan concentrations higher than 2 g L<sup>-1</sup> could be more efficient in reducing bacterial growth. The inhibitory effect of chitosan coatings on the growth of mesophilic bacteria had been broadly described (Chien et al., 2007; Gonzalez-Aguilar et al., 2008; Simões et al., 2009; Xu et al., 2013), however, only few information is available for psychrophilic bacteria. Campaniello et al. (2008) reported an important inhibitor effect on psychrotrophic microflora of minimally processed strawberries. This inhibition led to an appreciable prolongation of lag phase, a lower cell load and, consequently, an increase of the stability of the product.

The effect of chitosan on mould and yeasts development is shown in Table 3. Very low ( $< 2$  log CFU/gr) or undetectable initial numbers of yeast and mold counts were founded in coated or uncoated pear slices (data not shown). The counts steadily increased reaching about 2-3 log CFU/gr at the end of the storage, regardless of the use of coatings (Table 3). The high chitosan concentration was the only sufficiently efficient in controlling microbial growth.

The beneficial effect on yeasts and mould inhibition associated with the use of chitosan has been largely described (Rabea et al., 2003; Devlieghere et al., 2004; Zhang et al., 2011; Perdones et al., 2012). Numerous previous studies have shown that chitosan could directly inhibit spore germination, germ tube elongation and mycelial growth of many phytopathogens. Different mechanisms have been proposed to explain the mode of action of chitosan, however,

the exact mechanism of its antimicrobial action is still imperfectly known (Zhang et al., 2011).

### Conclusions

This study showed that chitosan coatings are useful for the preservation of fresh-cut 'Rocha' pear. Browning, tissue softening and microbial growth were efficiently reduced by chitosan coatings. The nutritional and sensorial attributes, as well as the external aspect of the product, were not negatively affected by the treatments. In conclusion, chitosan coatings could be a good alternative to extend the shelf-life of fresh-cut 'Rocha' pear, preserving the quality attributes and safety of the product.

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### Author contributions

C. S. wrote the manuscript. C. S. and F. C. L. made a major contribution to the review paper. C. S., F. C. L. and G. B. were involved in overall planning and supervision. M. V. executed all the lab experiments. P. R. and M. S. gave technical support.

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