Browning inhibition and microbial control in fresh-cut persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante) by apple pectin-based edible coatings

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Abstract

The aim of this study was to develop new edible coatings based on apple pectin with a combination of antioxidants and antimicrobial agents to control enzymatic browning and microbial growth of fresh-cut 'Rojo Brillante' persimmon. The survival of important food-borne human pathogens artificially inoculated on fresh-cut fruit was also assessed. Potassium sorbate (PS) at 2 or 4 g kg^{-1}, sodium benzoate (SB) at 4 g kg^{-1}, or nisin (NI) at 500 IU mL^{-1}, were added to apple pectin coatings containing 10 g kg^{-1} citric acid and 10 g kg^{-1} calcium chloride as antioxidants. Persimmon slices were dipped in the coatings, the aqueous antioxidant solution (citric acid and calcium chloride) or water (control), packed in an ambient atmosphere and stored at 5 ºC for up to 9 days. Microbial growth, colour, firmness, polyphenol oxidase (PPO) activity, visual quality and overall sensory flavour were measured during storage. Coated samples and those dipped in the antioxidant aqueous solution presented lower *a** values than control samples, which indicated effective browning inhibition. Persimmon slices treated with coatings containing PS and SB reached the limit of marketability after 7 days of storage. At the end of storage, the overall fruit flavour was ranked above the limit of acceptability. Antimicrobial coatings inhibited growth of mesophilic aerobic bacteria, and those containing SB and NI were the most effective. No growth of moulds, yeasts and psychrophilic aerobic bacteria was detected during storage. All the treatments effectively reduced the populations of *Escherichia coli* and *Salmonella enteritidis*, being the NI-coating the most effective. For *Listeria monocytogenes*, only the NI-coating effectively reduced the bacterial population.

Keywords: Minimally processed persimmon, edible coatings, antimicrobial agents, sensory and microbial quality, food-borne human pathogens, shelf life

1. Introduction

'Rojo Brillante' persimmon is an important cultivar in the Ribera del Xúquer area (Valencia, Spain). When harvested, it is an astringent variety, but the application of high CO_2 levels allows the removal of astringency without affecting fruit firmness (Salvador et al., 2007), which enables this fruit to be commercialised as a fresh-cut commodity. However, fruit processing promotes faster deterioration due to tissue damage, which leads to increased physiological activity and major physico-chemical changes, such as enzymatic browning, softening, etc. During processing, spoilage and pathogenic microorganisms can also contaminate the product surface, and the nutrients inside the fruit contribute to their growth. Post-processing contamination or recontamination of the surface of food products by these pathogens has led to recalls and outbreaks of food-borne illness (Reij and Den Aantrekker, 2004). Although the growth of human pathogens on the flesh of fresh fruits is thought to be limited due to acidity, recent studies have documented the exponential growth of *Escherichia coli* O157:H7*, Salmonella* spp. and *Listeria monocytogenes* on a variety of fresh-cut fruits (Alegre et al., 2010). Pathogen growth has also been demonstrated in non-acidic fruit, such as melon, watermelon, papaya and mango (Penteado and Leitao, 2004; Strawn and Danyluk, 2010).

In recent years, the use of edible coatings has emerged as a new, effective, and environmental-friendly alternative mean to extend the shelf life of many products, including fresh-cut fruits and vegetables, by providing a barrier to water loss and gas exchange. Furthermore, their functional properties may be enhanced by the addition of food ingredients, such as antioxidants and antimicrobials, to enhance appearance, integrity and microbial safety, among others (Valencia-Chamorro et al, 2011b). The basic ingredients of edible coatings are proteins, polysaccharides and lipids; whereas, active

ingredients include other Generally Regarded as Safe compounds (GRAS) and food-grade additives to meet international regulations that considers edible coatings as part of the food (EU Directive 98/72/EC, 1998; US FDA, 2006). In previous research works by our group, a pectin-based edible coating containing 10 g kg^{-1} citric acid (CA) and 10 g kg^{-1} calcium chloride ($CaCl_2$) proved effective among different polysaccharide coatings to control the enzymatic browning of fresh-cut 'Rojo Brillante' persimmon (unpublished data). This effect was attributed to the capability of pectin to form strong insoluble polymers upon the reaction with multivalent metal cations like calcium (Oms-Oliu et al., 2008a).

Microbiological stability is also a critical factor to maintain the commercial marketability of fresh-cut produce. Incorporating antimicrobial compounds into edible coatings is becoming an important practice for the potential development of novel treatments for fresh-cut fruits as it helps to reduce the deleterious effects of processing. The use of these substances has its advantages over the direct application of antibacterial agents onto foods because edible films can be designed to slow down the diffusion of antimicrobials from food surfaces. The effectiveness of different antimicrobial substances, such as lysozyme, nisin (NI), organic acids, essential oils and their derivatives incorporated into edible films against several pathogens has proven satisfactory (Rojas-Graü et al., 2009; Valencia-Chamorro et al., 2011b). Among them, essential oils are the most studied antimicrobial ingredients incorporated into edible coatings against pathogenic microorganisms in fresh-cut fruits. However, in many cases effective concentrations adversely affected the sensory properties of coated fruit (Rojas-Graü et al., 2009; Valencia-Chamorro et al., 2011b). On the other hand, potassium sorbate (PS), sodium benzoate (SB) and NI are widely used by the food industry as safe antimicrobial

food additives, although they have been less studied as edible coating ingredients to control microbial growth in fresh-cut fruits. Nevertheless, some studies have proved the antimicrobial activity of a cellulose-based edible coating amended with 1 g kg^{-1} SB or PS in fresh-cut apple and potato (Baldwin et al., 1996) and a starch-based coating containing 2 g L^{-1} PS on fresh strawberries under cold storage (García et al., 2001). A more recent work also reported that the application of cellulose films containing 7,500 IU mL^{-1} NI inhibited the growth of *Staphylococcus aureus* and *L. monocytogenes* in processed mangoes (Teixeira-Barbosa et al., 2013). However, no research studies about the effect of incorporating these compounds into pectin-based edible coatings applied to fresh-cut persimmon to ensure quality and safety have been published. Therefore, the aim of this work was to determine the effects of different antimicrobial agents, incorporated into an optimised apple pectin-based edible coating, on fruit quality and microbial growth of fresh-cut 'Rojo Brillante' persimmon. The survival of important food-borne human pathogens artificially inoculated on fresh-cut fruit was also assessed.

2. Materials and Methods

2.1. Plant material

Persimmons (*Diospyros kaki* Thunb cv Rojo Brillante) at commercial maturity were provided by a local packinghouse assigned to the persimmon geographical indication 'Denominación de Origen Kaki Ribera del Xuquer' (Valencia, Spain). Persimmons were harvested with an average external colour index (CI=1,000*a/L*b) of 13.29±3.17, firmness of 45.76±6.69 N, total acidity of 38.17±4.06 g of malic acid per 100 g and soluble solid content of 15.13±0.31 ºBrix. Before the experiments, fruit were selected for size and absence of physical damage and randomly divided into 6 groups,

which corresponded to 4 coating treatments, 1 antioxidant-dipped treatment, and 1 water-dipped control. The persimmons were free of any postharvest treatment.

2.2. Edible coatings formulation

Edible coatings were elaborated from a base solution of apple pectin (Sigma-Aldrich, St. Louis, MO, USA) at 10 g kg^{-1}. Aqueous solutions of apple pectin were prepared at mild heating. Glycerol (Panreac Quimica, S.A., Barcelona, Spain) was added as a plasticizer at 10 g kg^{-1}, and coating solutions were emulsified with 2.5 g kg^{-1} oleic acid (Panreac Quimica, S.A.) and 2.5 g kg^{-1} Tween 80 (Sigma-Aldrich). As antioxidant agents, 10 g kg^{-1} citric acid (CA; E-330) (Quimivita, Barcelona, Spain) and 10 g kg^{-1} calcium chloride ($CaCl_2$; E-509) (Sigma-Aldrich) were incorporated into the coating formulations. The antimicrobial agents tested were potassium sorbate (PS; E-202) at 2 or 4 g kg^{-1}, sodium benzoate (SB; E-211) at 4 g kg^{-1}, or nisin (NI; E-234) at 500 IU mL^{-1}. All these ingredients are classified as food additives (with their correspondent E-number) or GRAS compounds by the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (US FDA) and the concentrations tested were within the legal limit. PS and SB were supplied by Sigma-Aldrich Chemie (Steinheim, Germany) and NI was acquired from Coralim Aditivos S.L. (Valencia, Spain). Coating solutions were kept at 5 ºC until application.

2.3. Pathogenic strains and inoculum preparation

Stock cultures for the food-borne contamination-specific human pathogenic strains of *E. coli* serotype O157:H7 (CECT 4972; ATCC 700728), *Salmonella enterica subsp. enterica* (CECT 4300; ATCC 13076) and *L. monocytogenes* serovar 1 (CECT 7467;

ATCC 19111) were obtained from the Microbiology Reference Laboratory (University of Valencia, Spain) in the form of agar slants. Strains were activated by streaking on MacConkey's agar (AES Laboratoire, Combourg, France) (*E. coli* and *S. enteritidis*) and tryptic soya agar + 50 g kg^{-1} sheep's blood agar (BD, New Jersey, USA) (*L. monocytogenes*) plates, followed by incubation for 48 h at 37 °C. Single colonies were grown individually in Luria-Bertani broth (Luria-Bertani®, Barcelona, Spain) (*E. coli* and *S. enteritidis*) or tryptone soya yeast extract broth (Sigma-Aldrich Chemical Co., St. Louis, MA, USA) (*L. monocytogenes*) for 24 h at 37 ºC. Bacterial cells were harvested by centrifugation at 3,000 rpm for 10 min at 10 ºC and then resuspended in saline peptone to obtain a concentrated suspension. The process was repeated 3 times. Finally, cell pellets were resuspended in maximum recovery diluent to obtain a culture optical density of 0.2 at 600 nm. This corresponded to a final inoculum concentration of 6.0 log cfu mL^{-1}.

2.4. Persimmon processing and packaging

Natural astringency of 'Rojo Brillante' persimmons was eliminated by placing them for 24 h in closed chambers at 20 ºC with an atmosphere containing 95±2 kPa CO_2. Chambers used for deastringency consisted of hermetically sealed, transparent polymethyl methacrylate cabinets (82 x 62 x 87 cm) fitted with outlet and inlet ports through which CO_2 (Alphagaz, Air Liquide España S.A., Madrid, Spain) were injected until the desired concentration was achieved. The cabinets were also fitted with internal basal water trays that allowed achieving a high relative humidity (RH of 95 ± 5%). CO_2 level, temperature, and RH were continuously monitored by means of the computer-controlled system (Control-Tec®, Tecnidex S.A., Paterna, Valencia, Spain). After removal from the chambers, the fruit were stored in air at 5 ºC for 1 day until processing.

Persimmons were sanitised in a 150 mg L^{-1} NaClO solution for 2 min, rinsed with tap water, and dried prior to the cutting operations. For the physico-chemical, sensory and microbiological analyses, persimmons were peeled, cut into eight wedges and dipped into the pectin-based coatings for 3 min. As controls, fruit wedges were dipped for 3 min in water or in the aqueous antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$). After dipping, persimmon pieces were removed and left to dry at 5 ºC.

Six persimmons at the most were processed at the same time to minimise excessive exposure to oxygen. A sharp stainless-steel knife was used throughout the process to reduce mechanical bruising. Samples were processed in a temperature-controlled room at 5±1 ºC to avoid breaking the cold chain during the trials. Four persimmon pieces (115 ± 10 g) were placed onto polypropylene trays (17.4 x 12.9 x 3.6 cm, 470 ml, Ilpra Systems, Barcelona, Spain) and sealed with microperforated polypropylene film (64-µm thickness; ILPRA Systems) as secondary packaging. A total of 9 trays per treatment and sampling time were prepared that corresponded to 3 trays for physico-chemical analysis, 3 trays for sensory and 3 trays for microbiological analysis. To ensure that the surrounding atmosphere on the tray remained unchanged, the film was further perforated with a needle (four perforations, 1 mm in diameter). During storage, the gas composition in the package headspace was monitored with an O_2/CO_2 analyzer to verify that no changes in the headspace gas composition occurred (CheckMate 3, PBI Dansensor Inc., Denmark). Nine trays were prepared per treatment and sampling period to determine physico-chemical, sensory and microbial quality. Samples were stored up to 9 days at 5 ºC.

2.5. Microbiological growth in fresh-cut persimmons

On days 0, 4 and 8 of cold storage, the total amounts of mesophilic and psychrophilic aerobic bacteria, yeasts and moulds were determined according to the International Standard Organization Norms (ISO 17410:2001; ISO 21527:2008; ISO 4833-1:2013). A representative sample of persimmon wedges (10 g) was removed aseptically from the packaging, transferred to a sterile plastic bag and blended for 2 min with 90 mL of phosphate buffer (pH=7) in a homogenizer (Stomacher®400, Seward Ltd., Worthing, UK). Serial dilutions were prepared using sterile phosphate buffer. Then 0.1 mL was plated onto plate count agar (PCA) (Sigma-Aldrich Chemical Co., St. Louis, MA, USA). Duplicate plates were incubated for 2 days at 35 ºC and 10 days at 7 ºC to enumerate mesophilic and psychrophilic aerobic bacteria, respectively. For molds and yeasts, 0.1 mL of the dilutions was spread onto potato dextrose agar (PDA) (Sigma-Aldrich Chemical Co., St. Louis, MA, USA) and incubated for 5 days at 25 ºC. After incubation, colonies were enumerated and the results were expressed as $\log_{10}$ cfu per g of persimmon. Three trays per treatment and sampling time were analysed, which corresponded to 3 replicates.

2.6. Bacterial population of inoculated food-borne human pathogens on fresh-cut fruits

For the pathogenic analysis, persimmons were cut into slices and plugs of 1.2 cm in diameter, 1 cm long (weighting approx. 1 g) using a cork borer, to achieve a uniform inoculation of the samples (Alegre et al., 2010). Persimmon plugs were inoculated by immersion in the mixed bacterial inoculum (6 $\log_{10}$ cfu g^{-1}) for 2 min. Once dried, plugs were immersed for 3 min in the pectin-based edible coatings, the antioxidant aqueous solution or in water as a control, dried in a flow cabinet to avoid contamination of the

samples, and packed as described above. For each pathogen and sampling time, 3 polypropylene trays were prepared per treatment, containing 18 plugs each.

The concentrations of *E. coli*, *S. enteridtidis* and *L. monocytogenes* on persimmon plugs were determined before (BT) and after (AT) treatment, and also after 4 and 8 days at 5 ºC according to the ISO Norms (ISO 11290-2:1998; ISO 6579:2002; ISO 7251:2005). At each sampling time, 10 g of inoculated persimmon, which corresponded to 9-10 plugs, were placed into sterile plastic bags and 90 mL of phosphate buffer (pH=7) were added. The mixture was homogenised in the blender (Stomacher®400) for 2 min. Serial dilutions were made using sterile phosphate buffer and 100 µL were then pour plated onto the corresponding plates. Counts of *E. coli* and *S. enteritidis* were done in MacConkey's agar after incubation at 37 ºC for 24 and 36 h, respectively. Counts of *L. monocytogenes* were done in tryptic soya agar, plus 50 g kg^{-1} sheep's blood agar, after incubation for 2-3 days at 37 ºC. There were 3 replicates per treatment for each pathogen and sampling time and each assay was also repeated 3 times. The results were expressed as $\log_{10}$ cfu g^{-1}.

2.7. Colour evaluation

Fresh-cut fruit colour (CIELAB parameters *L**, *a**, and *b**) was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) using 12 pieces of persimmon per treatment. Each measurement was performed randomly at three different locations per sample piece. A standard white calibration plate was employed to calibrate the equipment. Results were expressed as the average colour of 12 samples per treatment.

2.8. Firmness measurements

The firmness of the fresh-cut persimmons was evaluated in an Instron Universal Machine (Model 3343, Instron Corp., Canton, MA, USA) by measuring the force required for an 8-mm diameter rod to penetrate the sample to a depth of 2 mm and at a speed of 5 mm s^{-1}. Twelve samples per treatment were measured and the results were expressed in N.

2.9. Polyphenol oxidase (PPO) activity

For enzyme extraction, 15 g of fresh persimmon were blended and mixed with McIlvaine buffer solution (1:1) at pH 6.5, containing 1 mol L^{-1} sodium chloride and 50 g kg^{-1} polyvinylpolypyrrolidone (Ultra-Turrax, IKA, Staufen, Germany). Then, the homogenate was centrifuged at 12,000 rpm at 4 ºC for 30 min. The supernatant was collected to its activity measurement. Two extractions were taken per each replicate.

To determine enzyme activity, 3 mL of 0.05 mol L^{-1} 4-methylcatechol was added to 100 µL of the enzyme extract in a 4.5 mL quartz cuvette of a 1 cm path length. The changes in absorbance were determined every 5 s at 420 nm for up to 2 min from the time the enzyme extract was added in a spectrophotometer (UV-1, Thermo Electron Corporation, UK). Three replicates per treatment were measured. Activities were expressed in absorbance per min. All the reagents used were obtained from Sigma (St. Louis, MO, USA).

2.10. Sensory quality

During storage, persimmon slices were evaluated visually by 15 trained judges. Each treatment was presented to panellists on trays that contained 12 persimmon pieces to account for sample variability, and labelled with a 3-digit random code. Visual quality,

based on general visual appearance, was determined by the following visual scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny et al., 2002). A colour photograph of the samples rated with this scale was used by the judges to score samples.

The panellists also evaluated off-flavours, firmness and the overall flavour of the fresh-cut 'Rojo Brillante' persimmon pieces. Off-flavour was rated on a 5-point scale, where 1=absence and 5=marked presence. Firmness was rated on a 5-point scale, where 1=very soft and 5=very firm. Overall flavour was rated on a 9-point scale, where 1 to 3 represented a poor quality range, 4 to 6 an acceptable quality range, and 7 to 9 an excellent quality range. These attributes were evaluated in 2 persimmon slices randomly selected from each treatment to compensate for the biological variation of material. They were presented to the panelists on trays labelled with the 3-digit codes and were served at room temperature (25±1 ºC). Spring water was used for palate cleansing between samples. To avoid discrimination due to colour, samples were illuminated with appropriate lighting to completely mask browning.

2.11. Statistical analysis

The statistical analysis was performed by STATGRAPHICS 5.1 (Manugistics, Inc., Rockville, Maryland, USA). Specific differences among treatments were determined by the least significant difference (LSD) test when the analysis of variance (ANOVA) showed a significant *F*-value. Significant differences were defined at $P \leq 0.05$.

3. Results and Discussion

3.1. Microbial growth in fresh-cut persimmon

Under the studied conditions, growth of molds, yeasts and aerobic psychrophilic bacteria was not observed during storage at 5 ºC in all fresh-cut persimmons, including the control samples dipped in water (data not shown). However, the counts of total aerobic mesophilic bacteria significantly increased in control samples during storage (Fig. 1). Immersion in the antioxidant solution or pectin-based coatings effectively maintained or reduced the growth of mesophilic bacteria. The application of 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ in aqueous solution was as effective as the pectin-based edible coatings amended with 2 or 4 g kg^{-1} PS. Only the addition of 500 IU mL^{-1} NI or 4 g kg^{-1} SB to the pectin-based coating showed a synergic effect with the antioxidant solution, and totally inhibited aerobic mesophiles by storage day 4 and 8, respectively.

'Rojo Brillante' persimmon is described as a low acidity fruit with a pH around 6 (Salvador et al., 2007). Therefore, it can be considered a fruit with high microbial risk (pH>4.5) and the reduction of the pH would contribute to reduce microbial growth. Evidence of the antimicrobial properties of organic acids like citric, sorbic, benzoic, lactic or oxalic acids, and organic acid salts like PS and SB, can be frequently found in the literature (Valencia-Chamorro et al., 2011b). Their antimicrobial activity has been attributed to pH reduction, depression of the internal pH of microbial cells by the ionisation of undissociated acid molecules, and disruption of substrate transport by altering cell membrane permeability (Beuchat, 1998). Calcium ion, which mode of action is mainly associated with maintaining cell wall structure and firmness, has also been reported to stunt microbial growth in fresh-cut commodities, such as melon (Aguayo et al., 2008), nectarine (Cefola et al., 2014), or papaya (Waghmare and Annapure, 2013), among others. The addition of the additives CA, ascorbic acid or $CaCl_2$ as antioxidant/firming agents to polysaccharide coatings like alginate, carrageenan,

cellulose, xanthan gum or gellan, has also been reported to confer antimicrobial activity to coatings and help control microbial growth in fresh-cut apples and pears (Lee et al., 2003; Rojas-Graü et al., 2008; Freitas et al., 2013). Regarding organic acid salts, optimal antimicrobial activity has occurred at low pH values, when the undissociated form is present. In our research work with pectin-based coatings, the addition of CA lowered the pH of the formulations to pH values of 2.6, which fall within the optimum range for the antimicrobial activity of these organic acid salts (Valencia-Chamorro et al., 2011a). Similar results have been reported by Baldwin et al. (1996), who found that the adjustment of a cellulose-based edible coating, amended with SB and PS, to pH of 2.5, provided an optimal microbial control in fresh-cut apple and potato. Activity of nisin, which is known to destabilise the cytoplasmic membrane of bacteria via an electrostatic interaction when contact is produced, was also enhanced at low pH (Ross et al., 2003).

3.2. Bacterial population of inoculated food-borne human pathogens on fresh-cut persimmons

The effect of pectin-based coatings on the growth of *E. coli*, *S. enteritidis* and *L. monocytogenes* in artificially inoculated fresh-cut 'Rojo Brillante' persimmons is shown in Fig. 2. The application of coatings (AT application) lowered the initial *E. coli* populations by more than 1.0 $\log_{10}$ units depending on treatments, and NI was the most effective antimicrobial. After 4 days of storage at 5 ºC, the *E. coli* population was significantly reduced in samples treated with coatings containing PS or SB at 4 g kg^{-1} and NI. At the end of storage, only the coatings containing antimicrobials maintained the reduction of 1.0 $\log_{10}$ units in the *E. coli* population present in persimmon plugs, whereas the population in control (water) and antioxidant-treated samples increased by about 2.0

log_{10} units. In the case of *S. enteritidis*, the initial population levels in the water-control persimmons were maintained during the storage period, whereas the other treatments effectively reduced the population throughout storage at 5 ºC. On storage day 8, the samples dipped in the antioxidant aqueous solution and those coated with the pectin-NI coating showed the lowest population values, with a reduction of 2.0 log_{10} units from the initial values. The population of *L. monocytogenes* was reduced only in the samples dipped in the pectin-NI edible coating, this reduction being 4.0 log_{10} units after coating application (AT) and maintained during the entire 8-day storage period. Conversely, while an increase in the growth of this pathogen was observed in the control samples during storage, the initial counts were maintained in samples subjected to the other treatments.

The results indicate that the addition of NI to pectin-based edible coatings effectively reduced or maintained the populations of *E. coli, S. enteritidis* and *L. monocytogenes*. The effectiveness of NI inhibiting Gram-positive bacteria, including *L. monocytogenes*, is well-known, whereas it has little or no effect on Gram-negative bacteria such as *E. coli* or *Salmonella* spp. (Valencia-Chamorro et al., 2011b). The effectiveness of NI alone against pathogenic microorganisms in fresh-cut fruits is scarcely indicated in the literature. However, Teixeira-Barbosa et al. (2013) reported that the application of cellulose films containing 7,500 IU mL^{-1} NI inhibited the growth of *S. aureus* and *L. monocytogenes* in processed mangoes. In general in this work, the activity of NI against *L. monocytogenes* increased at low pH. In whey protein-based films, the joint use of NI with malic or citric acid as formulation ingredients exerted a synergic effect against *Listeria* spp., which was attributed to the formation of pores in the bacterial cell membrane by NI, thus facilitating the penetration of the acids (Pintado et al., 2009).

Some studies have also shown that the combination of NI with chelating agents, such as EDTA or certain acids, can improve the bactericidal effect towards both Gram-positive and Gram-negative bacteria (Stevens et al., 1991; Ukuku and Fett, 2004). For instance, in fresh-cut cantaloupe melon, the combination of NI (50 μg mL^{-1}), EDTA (0.02 mol L^{-1}), sodium lactate (20 g L^{-1}), and PS (0.2 g L^{-1}) reduced the population of *Salmonella* sp. by 1.4 log cfu/g (Ukuku and Fett, 2004). Therefore, the effectiveness in our work of NI-formulated coatings against the three considered food-borne pathogens might be related to the combination of NI with CA and a subsequent pH reduction in the coating.

3.3. Colour and PPO activity of fresh-cut persimmon

In general, increased enzymatic browning of persimmon pieces during storage was accompanied by an increase in *a** and a decrease in *L**, *b** and hue values (Fig. 3). The lowest *b** and hue values, and the highest *a** value were obtained in control samples dipped in water. The coatings containing PS or SB at 4 g kg^{-1} significantly decreased the lightness (*L**) of persimmon slices, whereas no significant differences were observed during storage among fruit subjected to the other treatments. Coating application and dipping in the antioxidant aqueous solution helped maintain *a** values during storage, although the most effective coating was that containing 2 g kg^{-1} PS. Changes in hue angle during storage confirmed the results observed for *a** values, i.e. the coating that contained 2 g kg^{-1} PS was the most effective to maintain high hue angle values in persimmon tissue over 7 storage days at 5ºC. Overall, this coating maintained lower *a** and higher hue values than the antioxidant aqueous solution, which indicates its potential to control the enzymatic browning of fresh-cut persimmons.

Several works have reported the effectiveness of edible coatings to control the enzymatic browning of fresh-cut fruits and vegetables when antioxidants are incorporated into base formulations. The effect of coatings on browning control greatly depends on intrinsic factors such as the polymer, the antioxidant compound and the fresh-cut commodity. For example, pectin-based coatings with N-acetylcysteine and glutathione added as antioxidants significantly reduced the browning of fresh-cut pears (Oms-Oliu et al., 2008b). Likewise, in preliminary work conducted by our group, a pectin-based edible coating containing 10 g kg^{-1} CA and 10 g kg^{-1} $CaCl_2$ proved to be more effective to control the enzymatic browning of fresh-cut persimmon than soy protein isolate or hydroxypropyl methylcellulose-based coatings, which had been amended with the same antioxidants (unpublished data). This effect was attributed to pectin capability to form strong insoluble polymers upon the reaction with multivalent metal cations like calcium, as discussed by Rhim (2004). In the present work, the addition of antimicrobials to the antioxidant pectin-based coatings affected some colour parameters of the cut persimmon pieces, which reflects the importance of minor ingredients for the final performance of coatings. Thus, the addition of 2 g kg^{-1} PS helped maintain lower a^* values (lesser browning) than the antioxidant aqueous solution, which suggests a synergic effect of its active form (sorbic acid) with antioxidants. However, the incorporation of both organic acid salts at a higher concentration (4 g kg^{-1}) negatively affected the lightness of the cut surface.

PPO has been considered the main enzyme related to loss of quality of fresh-cut products since its activity is directly related to enzymatic browning. Therefore, controlling enzymatic browning has traditionally focused on reducing PPO activity by using anti-browning agents. The mechanism of enzymatic inhibition differs vastly for

each type of anti-browning agent. In particular, acidulants such as CA act by lowering pH below the optimum pH for PPO activity. In persimmon, optimum PPO activity has been reported to fall within a pH range of 5.5-7.5, depending on the substrate (Núñez-Delicado et al., 2003; Özen et al., 2004). Thus, after both *in vitro* and *in vivo* studies, Ghidelli et al. (2013) reported that among a wide range of antioxidants, CA was one of the most effective to control the browning of 'Rojo Brillante' persimmon through different mechanisms of action. One of these mechanisms was the reduction of pH below 4. In the same study, $CaCl_2$ also reduced flesh browning of fresh-cut persimmon, but to a lower extent than CA, which could be attributed to PPO inhibition by the chloride ion. In the present work, the application of the antioxidant aqueous solution and different coatings amended with antioxidants significantly reduced PPO activity in the fruit if compared to the control water-dipped samples (Fig. 4). In the control samples, PPO activity also increased with storage time to reach values above 0.03 (ΔA_{420} min^{-1} mL^{-1}) after 9 days of storage at 5 ºC, whereas those treated with antioxidants (either alone or incorporated into the pectin-based coating) obtained and maintained an average value below 0.02 (ΔA_{420} min^{-1} mL^{-1}). These results were in agreement with colour changes in the samples during storage (Fig. 3). In general, the incorporation of the different antimicrobial agents did not negatively affect PPO activity in the samples.

3.4. Firmness of fresh-cut persimmon

Loss of fruit firmness after inadequate or prolonged cold storage is one of the most limiting factors that affect the quality and consumer acceptability of 'Rojo Brillante' persimmon because this is a chilled sensitive cultivar (Salvador et al., 2007). Therefore, a high degree of flesh firmness at harvest appears as a crucial factor to maintain good

quality of minimally processed persimmons during processing and storage at 5 ºC. In this work, fruit firmness decreased from an initial value of 41.22±1.61 N to an average value of 18.94±0.77 N by the end of storage, and this reduction of tissue firmness was not affected by the application of coatings or the antioxidant solution (Fig. 5). Some authors have reported the effectiveness of adding $CaCl_2$ to coatings to retain the firmness of fresh-cut fruit (Olivas et al., 2003; Rojas-Graü et al., 2008). However, in our work, neither the pectin-based edible coatings nor the antioxidant aqueous solution containing 10 g kg^{-1} $CaCl_2$ improved fruit firmness compared to control samples. In previous research conducted by our group, although the use of $CaCl_2$ alone did not prove effective to preserve loss of firmness of fresh-cut persimmon, the combination of 10 g kg^{-1} $CaCl_2$ with 10 g kg^{-1} ascorbic acid or 1 g kg^{-1} CA prevented excessive softening of fresh-cut persimmons treated with acidic solutions, and helped maintain the firmness of the persimmon slices within the same range as the control samples (Sanchís et al., 2015). Similar results had been previously reported by Lee et al. (2003), who observed that the addition of $CaCl_2$ to an acidic dipping solution minimised the softening of apple slices.

3.5. Sensory quality of fresh-cut persimmon

Browning of fresh-cut persimmons treated with antioxidants was also assessed by a sensory panel to determine whether the colour differences instrumentally measured with the colorimeter can also be visually detected to the naked eye. The judges scored all the coated and antioxidant-treated samples within the limit of marketability after 7 days of storage at 5ºC, whereas the water-control samples were evaluated below this limit after 2 days of storage (Fig. 6). By day 7, the persimmon slices coated with the pectin-based coatings containing 2 g kg^{-1} PS or 4 g kg^{-1} SB were evaluated better than those containing

NI or subjected to the aqueous antioxidant treatment. Only the pectin-2 g kg^{-1} PS coating was evaluated to be near the limit of marketability by day 9, which can be related to the fact that among all treatments these samples presented the lowest *a** and the highest hue values (Fig. 3).

The incorporation of antioxidants and antimicrobials into pectin coatings conferred slight acidity to samples, as reported by the judges, which did not correspond to the typical persimmon flavour. These samples were evaluated as presenting a very slight 'off-flavour' (Table 1). Nevertheless, few or no differences were observed between the samples treated with the antioxidant solution and the coatings, which indicated that CA and $CaCl_2$ also contributed to this sensory perception to some extent. By the end of storage, only the persimmon slices treated with the pectin-4 g kg^{-1} SB coating obtained a higher score for the 'off-flavour' than the remaining samples, while no differences were found among the other treatments and the controls. Despite these results, the presence of antioxidants and antimicrobials slightly affected the overall flavour of treated samples and, by the end of the storage period, only the samples treated with 4 g kg^{-1} SB obtained the lowest score, while no differences were observed among the remaining treatments. All the treatments were generally evaluated within the limit of acceptability (5-6 range) during the whole storage period.

The sensory firmness evaluation confirmed the results obtained with the instrumental texture analysis (Table 1; Fig. 5). At the time of processing, persimmon slices were evaluated by the judges as being very firm. On storage day 2, persimmon pieces were evaluated as being firm and the values were maintained next to this range for all 9 storage days.

4. Conclusions

Antimicrobial pectin coatings and antioxidant aqueous solution significantly controlled enzymatic browning and reduced the total aerobic mesophilic bacteria of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC, which accomplished a commercial shelf life of 7 days. Overall, the coatings containing 2 g kg^{-1} PS or 4 g kg^{-1} SB proved to be the most effective to maintain the visual quality of persimmon slices. The combination of antioxidants with 500 IU mL^{-1} NI or 4 g kg^{-1} SB as coating ingredients also completely inhibited the growth of mesophilic aerobics in fresh-cut 'Rojo Brillante' persimmon after 4 and 8 days of cold storage, respectively. The use of coatings formulated with the combination of the antioxidant and 500 IU mL^{-1} NI also effectively stunted the growth of *E. coli, S. enteritidis* and *L. monocytogenes* in artificially inoculated fresh-cut persimmons.

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	Treatment	Storage days			
		2	5	7	9
Off-flavour	Pectin-2 g kg^{-1} PS	2.58 ± 0.38ab	1.88 ± 0.24ab	1.94 ± 0.27abc	1.86 ± 0.29ab
	Pectin-4 g kg^{-1} PS	2.58 ± 0.36ab	1.44 ± 0.27bc	1.47 ± 0.18bc	1.64 ± 0.29b
	Pectin-4 g kg^{-1} SB	2.67 ± 0.26ab	2.13 ± 0.30a	2.65 ± 0.27a	2.57 ± 0.29a
	Pectin-500 IU mL^{-1} NI	3.17 ± 0.38a	1.88 ± 0.27ab	1.82 ± 0.24bc	1.86 ± 0.24ab
	Antioxidant solution	1.92 ± 0.36bc	1.81 ± 0.28abc	2.18 ± 0.22ab	1.50 ± 0.33b
	Control	1.33 ± 0.41c	1.19 ± 0.23c	1.24 ± 0.22c	1.50 ± 0.33b
Flavour	Pectin-2 g kg^{-1} PS	5.08 ± 0.66ab	5.06 ± 0.32b	5.47 ± 0.40bc	5.43 ± 0.23a
	Pectin-4 g kg^{-1} PS	4.25 ± 0.66ab	6.00 ± 0.32ab	6.35 ± 0.34ab	5.43 ± 0.43a
	Pectin-4 g kg^{-1} SB	4.00 ± 0.52b	5.13 ± 0.48b	4.47 ± 0.45c	4.07 ± 0.45b
	Pectin-500 IU mL^{-1} NI	3.92 ± 0.51b	5.31 ± 0.38b	5.59 ± 0.39b	5.36 ± 0.39a
	Antioxidant solution	4.67 ± 0.57ab	6.00 ± 0.37ab	5.35 ± 0.36bc	6.29 ± 0.33a
	Control	5.92 ± 0.62a	7.00 ± 0.26a	6.71 ± 0.33a	5.93 ± 0.33a
Firmness	Pectin-2 g kg^{-1} PS	3.83 ± 0.21a	3.19 ± 0.21a	2.88 ± 0.22ab	3.36 ± 0.17a
	Pectin-4 g kg^{-1} PS	3.33 ± 0.19a	3.31 ± 0.18a	3.12 ± 0.19ab	2.57 ± 0.23c
	Pectin-4 g kg^{-1} SB	3.42 ± 0.23a	3.19 ± 0.14a	2.88 ± 0.22ab	2.86 ± 0.21abc
	Pectin-500 IU mL^{-1} NI	3.75 ± 0.25a	3.50 ± 0.18a	2.82 ± 0.20b	2.64 ± 0.17bc
	Antioxidant solution	3.67 ± 0.28a	3.38 ± 0.20a	3.35 ± 0.21ab	3.36 ± 0.20a
	Control	3.50 ± 0.34a	3.56 ± 0.20a	3.41 ± 0.17a	3.21 ± 0.24ab

Table 1. Sensory quality of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days.

PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride.
Off-flavour was rated on a 5-point scale, where 1=absence and 5=marked presence. Flavour was rated on a 9-point scale, where 1 = very poor quality and 9 = excellent quality. Firmness was rated on a 5-point scale, where 1=very soft and 5=very firm.
In each column, small letters indicate significant differences among treatments ($P \leq 0.05$).
Shown data are mean ± standard error.

Figure captions

Fig. 1 Growth of aerobic mesophilic bacteria on fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored for 8 days at 5 ºC. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical bars show standard error

Fig. 2 Populations of *Escherichia coli* (A), *Salmonella enteritidi*s (B) and *Listeria monocytogenes* (C) on artificially inoculated fresh-cut 'Rojo Brillante' persimmon plugs before (BT) and after (AT) dipping in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and after storage at 5 ºC for 4 and 8 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical bars show standard deviation

Fig. 3 Flesh color of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical bars show standard error

Fig. 4 Polyphenol oxidase (PPO) activity in fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical bars show standard error

Fig. 5 Firmness of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical bars show standard error

Fig. 6 Visual quality of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Visual scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible. Vertical bars show standard error

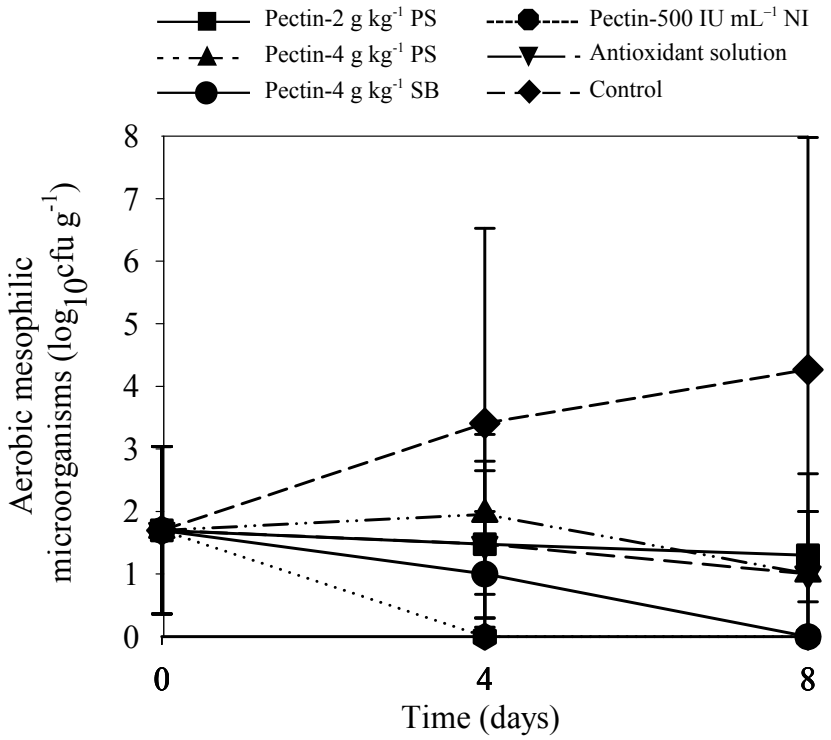
Pectin-2 g kg-1 PS
Pectin-500 IU mL−1 NI
Pectin-4 g kg-1 PS
Antioxidant solution
Pectin-4 g kg-1 SB
Control
Aerobic mesophilic microorganisms (log10cfu g-1)
0
1
2
3
4
5
6
7
8
0
4
8
Time (days)

Fig. 1.

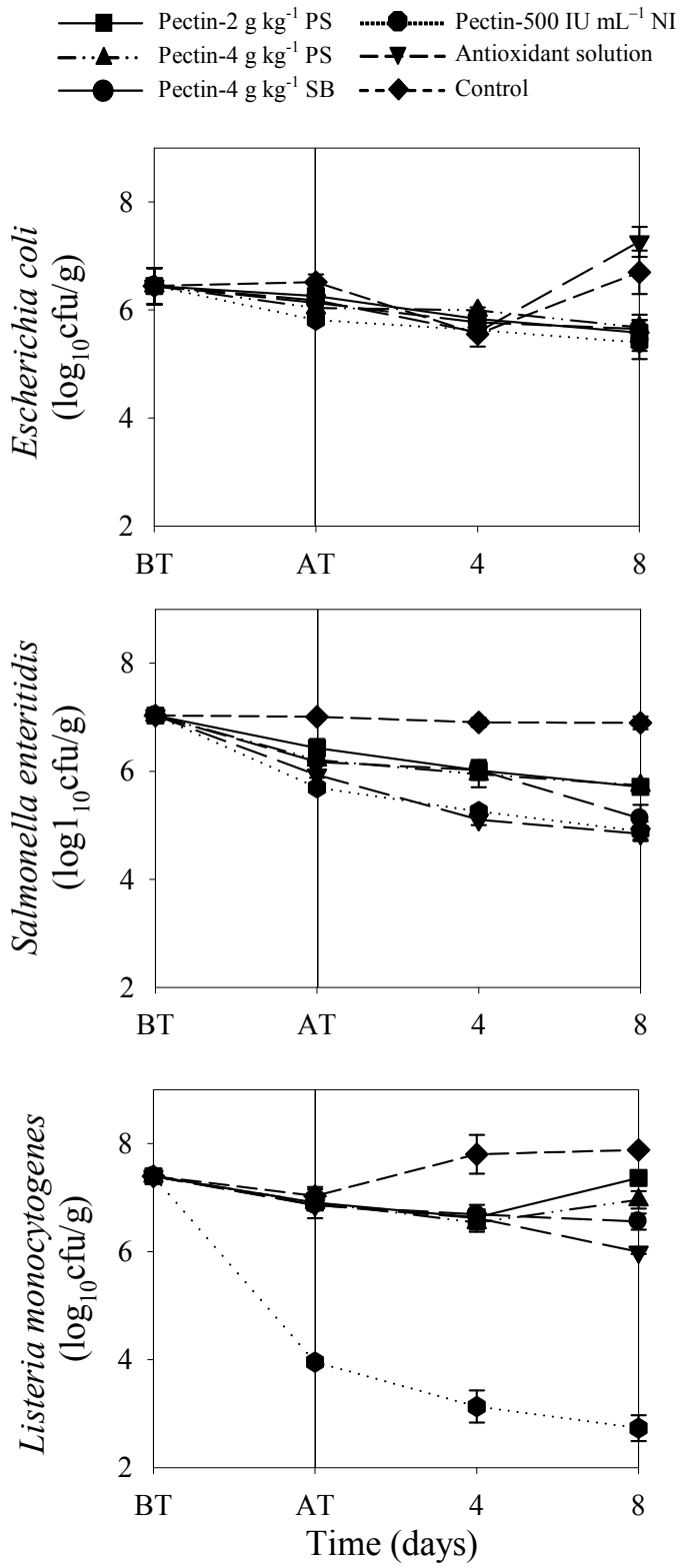
Pectin-2 g kg-1 PS
Pectin-4 g kg-1 PS
Pectin-4 g kg-1 SB
Pectin-500 IU mL−1 NI
Antioxidant solution
Control
Escherichia coli (log10cfu/g)
Salmonella enteritidis (log10cfu/g)
Listeria monocytogenes (log10cfu/g)
8
6
4
2
BT
AT
4
8
Time (days)

Fig. 2.

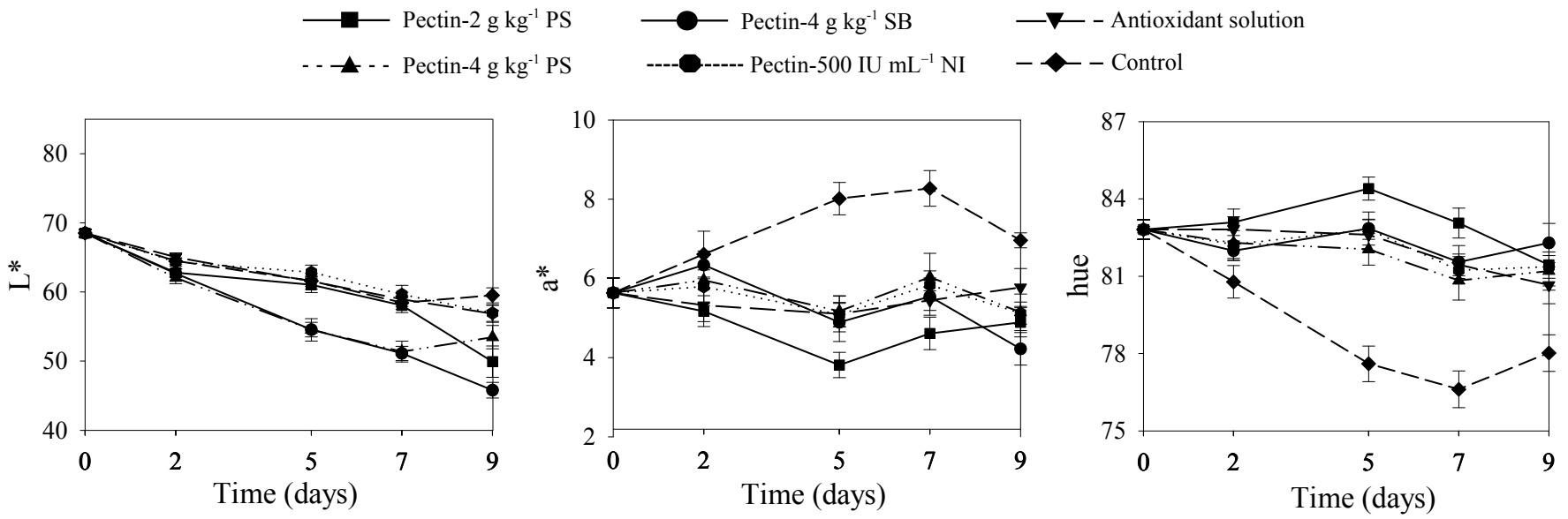
Pectin-2 g kg-1 PS
Pectin-4 g kg-1 SB
Antioxidant solution
Pectin-4 g kg-1 PS
Pectin-500 IU mL−1 NI
Control
L*
a*
hue
Time (days)
Time (days)
Time (days)

Fig. 3.

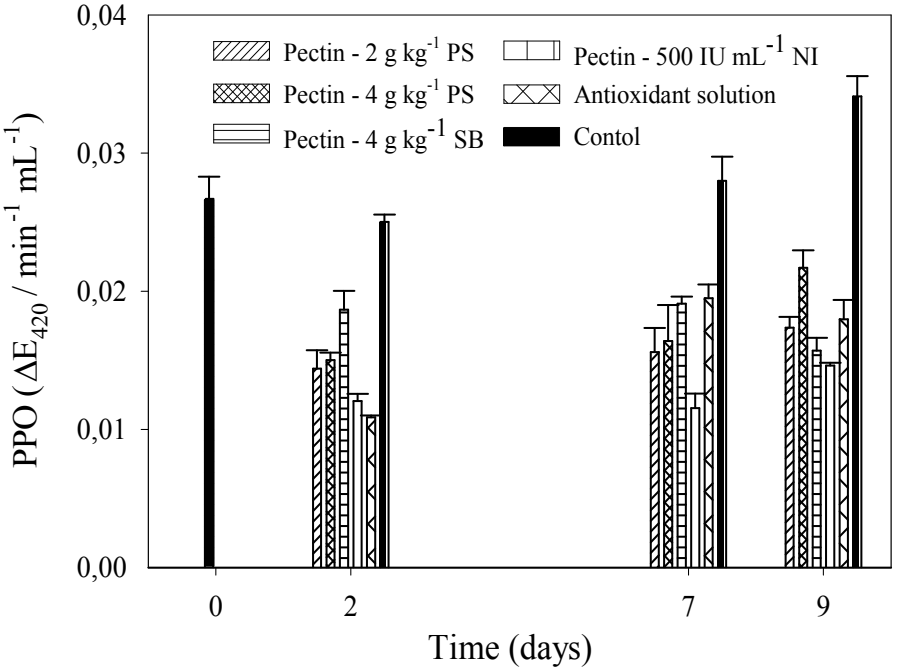
0,04
0,03
0,02
0,01
0,00
PPO (ΔE_{420} / min^{-1} mL^{-1})
Pectin - 2 g kg^{-1} PS
Pectin - 4 g kg^{-1} PS
Pectin - 4 g kg^{-1} SB
Pectin - 500 IU mL^{-1} NI
Antioxidant solution
Contol
0
2
7
9
Time (days)

Fig. 4.

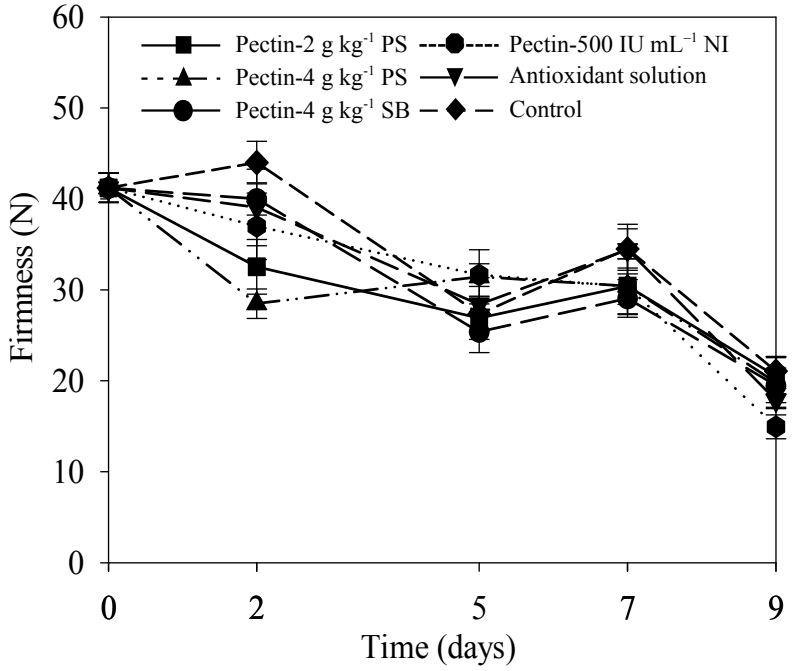
Pectin-2 g kg-1 PS
Pectin-500 IU mL-1 NI
Pectin-4 g kg-1 PS
Antioxidant solution
Pectin-4 g kg-1 SB
Control
Firmness (N)
Time (days)
0
10
20
30
40
50
60
0
2
5
7
9

Fig. 5.

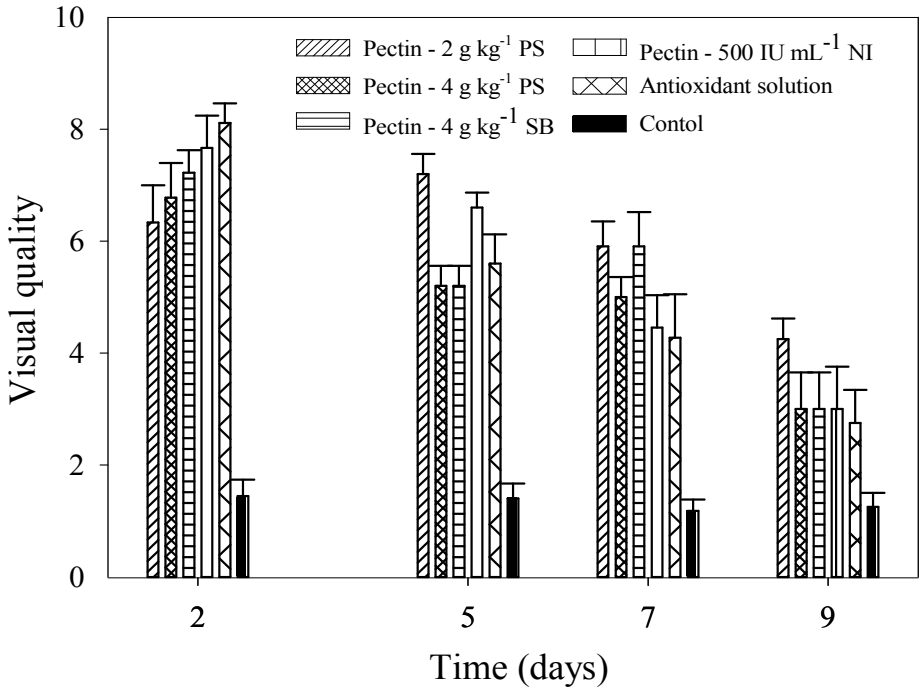
Pectin - 2 g kg-1 PS
Pectin - 4 g kg-1 PS
Pectin - 4 g kg-1 SB
Pectin - 500 IU mL-1 NI
Antioxidant solution
Contol
Visual quality
Time (days)
0
2
4
6
8
10
2
5
7
9

Fig. 6.