Integration of antimicrobial pectin-based edible coating and active modified atmosphere packaging to preserve the quality and microbial safety of fresh-cut persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante)

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Running title: Edible coating and active MAP to preserve quality and safety of cut persimmon

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Abstract

BACKGROUND: The greatest hurdle to the commercial marketing of fresh-cut fruits is related to their higher susceptibility to enzymatic browning, tissue softening, and microbial growth. The aim of this study was to test the efficacy of a pectin-based edible coating and low oxygen modified atmosphere packaging (MAP) to control enzymatic browning and reduce microbial growth of fresh-cut 'Rojo Brillante' persimmon. The survival of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* artificially inoculated on fresh-cut fruit was also assessed. The pectin coating was amended with 500 IU mL^{-1} nisin (NI) as antimicrobial agent and 10 g kg^{-1} citric acid and 10 g kg^{-1} calcium chloride as antibrowning and firming agents, respectively. Persimmon slices were dipped in the coating or in water (control) and packed under 5 kPa O_2 (MAP) or in ambient atmosphere for up to 9 days at 5 ºC. Microbial growth, package gas composition, colour, firmness, polyphenol oxidase (PPO) activity, visual quality and overall sensory flavour of persimmon slices were measured during storage.

RESULTS: Coating application combined with active MAP significantly reduced the CO_2 emission and O_2 consumption in the package. The coating was effective to reduce browning and also inhibited the growth of mesophilic aerobic bacteria. Coating also reduced the populations of *E. coli*, *S. enteritidis* and *L. monocytogenes*.

CONCLUSION: The combination of the pectin-based edible coating and active MAP proved to be the most effective treatment to maintain the sensory and microbiological quality of persimmon slices for more than 9 days of storage.

Keywords: Minimally processed persimmon, food-borne human pathogens, antibrowning agents, antimicrobial activity, shelf-life

INTRODUCTION

The demand for fresh-cut fruits and vegetables is continuously increasing, being the convenience factor and health promoting benefits associated with their consumption the main reasons for such an increament. 'Rojo Brillante' is the most important persimmon cultivar in Spain. This cultivar, mainly grown in the Ribera del Xúquer area (Valencia, Spain) has experienced in the last decade an important increase in planted surface and production due to the fruit good sensory characteristics and nutritional properties. When harvested, the fruit is astringent, but the exogenous application of high levels of CO_2 allows the removal of astringency without affecting fruit firmness, which enables this cultivar to be commercialized as a fresh-cut commodity. However, physical damage during peeling, cutting or slicing processes increases respiration rate, metabolic changes and susceptibility to microbial spoilage, which often result in degradation of the colour, flavour and firmness of the product.[1] Furthermore, cut surfaces can provide both attachment opportunities and entry points for microorganisms.[2] Recent studies have documented the exponential growth of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* in non acidic horticultural products as well as on a wide variety of acidic fresh produce, although the growth in the latest is thought to be limited because of the acidity.[3]

Main approaches to extend the shelf-life of fresh-cut products include chlorine sanitation, the use of low temperatures, modified atmosphere packaging (MAP) with low O_2 concentration, and the use of antioxidants and calcium salts. The effect of MAP to maintain the quality of fresh-cut products is related to a reduction in the product respiration rate, ethylene biosynthesis and action, water loss, phenolic oxidation, and aerobic microbial count.[4] However, the beneficial effects depend upon a number of uncontrollable factors, such as the species, cultivar, cultural practices, stage of maturity, as well as controllable factors, including packaging material gas permeability, respiration rate, and storage conditions.[5] Thus, previous work by our group showed that controlled atmosphere conditions with high CO_2 concentrations (10 or 20 kPa) induced in fresh-cut 'Rojo Brillante' persimmons the darkening of some tissue areas associated with a flesh disorder known as 'internal flesh browning'. The maximum fruit shelf life was achieved in samples stored in low O_2 atmospheres (5 kPa O_2, balance N_2), as this concentration effectively controlled enzymatic browning and prevented 'flesh browning'. Subsequent studies confirmed the beneficial effect of active MAP (5 kPa O_2) compared to passive MAP to improve the visual quality of fresh-cut 'Rojo Brillante' persimmon, showing a synergic effect with an antibrowning dip in citric acid and $CaCl_2$ (unpublished data).

Nowadays, edible coatings are gaining importance as an alternative treatment to reduce the deterioration caused by minimal processing fruits, as they provide a semipermeable barrier to gases and water vapour and, therefore, help to control respiration rate, enzymatic browning, and water loss. Furthermore, their protective function may be also enhanced by the addition of other ingredients such as antimicrobials, antioxidants, flavours, nutrients, etc.[6] It can be found in the literature numerous works remarking the effect of antioxidant edible coatings to control browning in fresh-cut fruits such as apple, pear, papaya, etc.[7-9] However, the incorporation of antimicrobial food additives into edible coatings to prevent microbial spoilage has been considerably less studied. In previous works by our group, the addition to a pectin-based edible coating of citric acid and $CaCl_2$, as antibrowning and firming agents, and nisin as antimicrobial agent effectively prevented enzymatic browning of fresh-cut 'Rojo Brillante' persimmon and extended the commercial visual shelf-life up to 8 days of storage at 5 ºC. In addition, the coating also inhibited the growth of mesophilic aerobics and reduced the population of inoculated *E. coli, S. enteritidis* and *L.* monocytogenes on cut persimmons.[10]

Since it is known that the application of hurdle technologies can considerably improve the overall quality of fresh-cut fruits and vegetables, some attempts have also been focused on extending the shelf life of fresh-cut commodities by combining both edible coatings and MAP technologies. For this instance, the use of MAP as a second technology resulted in a significant benefit on the visual quality of fresh-cut kiwifruit coated with a sodium alginate coating amended with grape seed extract,[11] and improved the microbiological quality of fresh-cut strawberries coated with chitosan.[12] In fresh-cut 'Rojo Brillante' persimmon, the combination of a soy protein isolate-based coating containing antioxidants with active MAP packaging (5 kPa O_2 + 15 kPa CO_2) showed a synergistic effect in controlling tissue browning and maintained the visual quality above the limit of marketability up to 8 days of storage at 5 ºC.[13] However, no studies are available on the effect of antimicrobial and antioxidant edible coatings combined with MAP on enzymatic browning and microbial quality of fresh-cut persimmons. Therefore, the aim of this work was to study the combined effect of a pectin-based edible coating amended with antibrowning and antimicrobial food additives and low O_2 MAP 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.

MATERIALS AND METHODS

Plant material

Persimmons (*Diospyros kaki* Thunb cv Rojo Brillante) harvested 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 external colour index (CI=1000 a /L b) of 15.1 ± 4.0, firmness of 21.4 ± 5.0 N, total acidity of 1.14 ± 0.02 g malic acid/100 g and a soluble solid content of 18.20 ± 0.09 ºBrix.

Edible coating formulation

The edible coating was elaborated from a base solution of apple pectin (Sigma-Aldrich, St. Louis, MO, USA) at 10 g kg^{-1}. The aqueous solution of apple pectin was prepared at mild heating. The pectin was emulsified with 2.5 g kg^{-1} oleic acid (Panreac Química, S.A., S.A., Barcelona, Spain) and 2.5 g kg^{-1} Tween 80 (Sigma-Aldrich) and glycerol (Panreac Química) was added as plasticizer at 10 g kg^{-1}. As antibrowning agents, 10 g kg^{-1} citric acid (Quimivita, Barcelona, Spain) and 10 g kg^{-1} calcium chloride ($CaCl_2$) (Sigma-Aldrich) were incorporated into the coating formulation. Nisin (NI) was added as antimicrobial agent at 500 international units (IU) mL^{-1} (Coralim Aditivos S.L., Valencia, Spain). The coating emulsion was kept at 5 ºC until application.

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), *S. 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) (*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}.

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 removing them from the chambers, fruit were stored in air at 5 ºC for 1 day until processing. Persimmons were sanitized in a 150 mg L^{-1} NaClO solution for 2 min, rinsed with tap water, and dried prior to cutting operations. For the physico-chemical, sensory and microbiological analyses, persimmons were peeled, cut into eight wedges with a sharp stainless-steel knife to reduce mechanical bruising and dipped into the pectin-based coating or in water as control for 3 min. After dipping, persimmon pieces were removed and left to dry at 5 ºC. Then, four persimmon pieces (115 ± 10 g) were placed on polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and sealed with 64-μm thickness, microperforated polypropylene-polyethylene terephthalate film (P12-2050PXNP, ILPRA Systems España S.L. Mataró, Spain). Oxygen and carbon dioxide permeance of the film were 110 and 500 mL m^{-2} d^{-1} bar^{-1} respectively, at 23 ºC. Coated and uncoated samples were divided into two groups. Half of the fruit was packed in air and the other half under active MAP of 5 kPa O_2 balanced with N_2. To ensure that the atmosphere on the trays that were packed in air was not modified, and to study the effect of only the edible coating, the film was perforated with a needle (4 perforations, 1 mm in diameter). 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. Samples were stored up to 9 days at 5 ºC.

Headspace gas composition

Gas composition (O_2 and CO_2) in the package headspace of fresh-cut persimmon were analyzed with a gas chromatograph (Trace GC, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector (TCD) and fitted with a Poropack QS 80/100 column (1.2 m x 0.32 cm i.d.). Temperatures for the oven, injector, and thermal conductivity detector were 35, 115, and 150 ºC, respectively. Helium was used as a carrier gas at flow rate of 22 mL min^{-1}. The gas sample was taken with a needle through an adhesive septum that had been stuck on the film. One milliliter of the gas headspace was injected into the system. O_2 and CO_2 concentrations were calculated using peak areas from standard gas mixtures of 15.0:2.5% O_2:CO_2. Results were expressed as kPa. Five trays per treatment were analyzed.

Microbial growth in fresh-cut persimmon

On days 0, 4 and 8, the total number of mesophilic and psychrophylic aerobic bacteria, yeasts and moulds was determined in triplicate. A representative sample of persimmon wedges (10 g) were removed aseptically from the package, 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 were plated onto plate count agar (PCA) (Sigma-Aldrich). Duplicate plates were incubated for 2 days at 35 ºC and 10 days at 7 ºC to enumerate mesophilic and psychrophylic aerobic bacteria, respectively. For moulds and yeasts, 0.1 mL of the dilutions were poured onto potato dextrose agar (PDA) (Sigma-Aldrich) and incubated for 5 days at 25 ºC. After incubation, colonies were counted and the results were expressed as $\log_{10}$ cfu per g of persimmon.

Populations of inoculated food-borne human pathogens on fresh-cut persimmon

For pathogenic analysis, persimmons were cut into slices and plugs of 1.2 cm of diameter, 1 cm long (weighting approx. 1 g) were prepared using a cork borer to achieve a uniform inoculation of the samples.[3] Persimmon plugs were inoculated by immersion in the bacterial inoculum (6 $\log_{10}$ cfu g^{-1}) for 2 min. Once dried, plugs were immersed for 3 min in the pectin-based edible coating or in water as control, dried in a flow cabinet to avoid contamination of the samples, and packed as described above (active MAP or air conditions).

The concentration of *E. coli*, *S. enteridtidis* and *L. monocytogenes* on persimmon plugs was determined just before (BT) and after (AT) the treatment (i.e. coating or water dips) and after 4 and 8 days at 5 ºC. At each sample time, 10 g of inoculated and treated plugs were placed into sterile plastic bags and 90 mL of phosphate buffer (pH=7) were added. The mixture was homogenized in a stomacher 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 made in MacConkey's agar after incubating at 37 ºC for 24 h and 36 h, respectively. Counts of *L. monocytogenes* were made in tryptic soy agar plus 5 % sheep's blood agar after incubating for 2-3 days at 37 ºC. There were three 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 per g of persimmon.

Colour evaluation

Colour (CIELAB parameters *L**, *a**, and *b**) was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) on 12 pieces of fresh-cut fruit per treatment. Each measurement was performed randomly at 3 different locations per sample piece. A standard white calibration plate was employed to calibrate the apparatus. The results were expressed as the mean of 12 samples per treatment.

Firmness measurements

The firmness of fresh-cut persimmon was evaluated using 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 at a speed of 5 mm s^{-1}. Twelve samples per treatment were measured and the results were expressed in newtons (N).

Polyphenol oxidase (PPO) activity

For the enzyme extraction, 15 g of fresh persimmon was blended and mixed with a McIlvaine buffer solution (1:1) at pH 6.5, containing 1 M sodium chloride and 5% polyvinylpolypyrrolidone (Ultraturrax, IKA, Germany). Then, the homogenate was centrifuged at 12,000 rpm and 4 ºC for 30 min. The supernatant was collected to its activity measurement. Two extractions were done per each replicate.

To determine enzyme activity, 3 mL of 0.05 M 4-methylcatechol was added to 100 µL of enzyme extract. The changes in absorbance were determined every 5 s in a spectrophotometer (UV-1, Thermo Electron Corporation, UK) at 420 nm for up to 2 min from the time the enzyme extract was added. Three replicates per treatment were measured. Activity was expressed in absorbance per minute. All the reagents used were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Sensory quality

During storage, persimmon slices were evaluated visually by 15 trained judges. Fruit from each treatment was presented to the panelists in 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 using 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.[14] A colour photograph of samples rated with this scale was used by the judges to score the samples.

The panellists also evaluated off-flavours, firmness and overall flavour of 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 in 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 the materials. The samples were presented to the panellists on trays labelled with the 3-digit codes and 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.

Statistical analysis

The statistical analysis was performed with the software Statgraphics 5.1 (Statpoint Technologies Inc., Warrenton, VA, USA). Specific differences among treatments were determined by the least significant difference (LSD) test when the analysis of variance (ANOVA) showed significant *P*-value. Significant differences were defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Headspace gas composition

Figure 1 shows the effect of the pectin-based edible coating on the content of O_2 and CO_2 inside packages of fresh-cut 'Rojo Brillante' persimmon under air and active MAP conditions. Samples packed in air maintained O_2 and CO_2 levels close to atmospheric values and no differences were observed between coated and uncoated samples. In contrast, in active MAP, the O_2 concentration decreased and reached the equilibrium by storage day 7, with values of 1 and 2 kPa, and the CO_2 concentration steadily increased during the 9 days of storage, with values of 6 and 7 kPa for coated and uncoated samples, respectively (P<0.05). Therefore, the effect of the pectin-based edible coating to reduce respiration rate of persimmon slices was only observed in samples packed under active MAP. Several studies have described the effect of polysaccharide-based edible coatings on reducing the respiration rate of fresh-cut products. For example, a depletion of respiration was reported in fresh-cut apple and melon dipped in an alginate-based edible coating when compared to uncoated samples [15,16] and in apple and mango slices coated with cassava starch.[17]

Microbial growth in fresh-cut persimmon

Growth of moulds, yeasts and aerobic psychrophilic bacteria was not observed during storage at 5 ºC in all fresh-cut persimmons, including control samples dipped in water (data not shown). Figure 2 shows the development of mesophilic bacteria on fresh-cut persimmon slices during cold storage at 5 ºC. The antimicrobial pectin-based edible coating effectively controlled the growth of mesophilic bacteria during storage independently of the packaging conditions; whereas bacterial growth increased in uncoated samples. In uncoated samples, no effect of the packaging condition was observed on storage day 4, with bacteria counts of 3.0 $\log_{10}$ cfu g^{-1}, but on day 8 these values increased to 4.0 log_{10} cfu g^{-1}, while they did not increased in persimmon slices packed in active MAP (*P*<0.05). The effect of low O_2 and high CO_2 concentrations on the growth of Gram negative bacteria, moulds, and aerobic microorganisms is well known. For example, packaging under active and passive MAP significantly inhibited the growth of spoilage microorganisms in fresh-cut pear, melon, honey pomelo, and mushroom slices, among others [8,18,19] and reduced the development of aerobic psycrhrotrophic bacteria and *Pseudomonas* in leaf spinach.[20] The effectiveness of MAP activity depends on the type and concentration of the microorganism, as well as on O_2 and CO_2 concentrations and ripeness stage of the commodity at processing. Oms-Oliu et al.[21] observed that active MAP (2.5 kPa O_2 + 7 kPa CO_2) inhibited bacterial growth, and yeast and mould proliferation in mature-green pears, but did not control microbial growth in partially ripe and ripe pears. In our case, the effect of active MAP on mesophilic bacteria was only observed in uncoated samples after 8 days of storage, when CO_2 concentration was close to 7 kPa and microbial population high.

In a previous work, a similar pectin-based edible coating amended with 500 IU mL^{-1} NI totally inhibited aerobic mesophiles in fresh-cut 'Rojo Brillante' persimmon.[10] NI has a broad activity spectrum against gram-positive bacteria, but do not significantly inhibit gram-negative bacteria, yeasts or moulds.[22] Activity of NI, which is known to destabilize the cytoplasmic membrane of bacteria via an electrostatic interaction when contact is produced, has been shown to be enhanced at low pH.[23] In our coating formulation, CA and $CaCl_2$ were added as antibrowning and firming agents and conferred a final pH to the formulation of 2.30. Furthermore, the use of the additives CA or $CaCl_2$ alone or incorporated to other edible coatings has also been reported to confer some antimicrobial activity in fresh-cut commodities such as fresh-cut apple and melon, which was related to their chelating activity.[24,25]

Populations of inoculated food-borne human pathogens on fresh-cut persimmon

The effect of the pectin-based edible coating and MAP on the growth of *E. coli*, *S. enteritidis* and *L. monocytogenes* in artificially inoculated persimmon plugs is shown in Figure 3. The application of the pectin antimicrobial coating significantly reduced the initial population of *E. coli* and *L. monocytogenes* by 1.5 and 1.0 $\log_{10}$ units (AT application), respectively, whereas *S. enteritidis* was reduced by more than 2.0 $\log_{10}$ units. The antimicrobial activity of the coating resulted in a further reduction of the population of the pathogens during storage at 5 ºC to achieve a complete inhibition of *E. coli* and *S. enteritidis* in the samples under MAP (Coating-MAP) or air conditions (Coating), respectively, by the end of the 8-day storage period. The population of *L. monocytogenes* in coated samples stored in MAP also dropped significantly, being more than 5.0 $\log_{10}$ units lower by the end of the storage period, whereas coated samples stored in air exhibited a slow decline in the population of *L. monocytogenes*, with only 2.0 $\log_{10}$ units reduction.

These results revealed the potential growth-inhibition effect of NI added to the pectin-based edible coating in order to reduce populations of these pathogens in fresh-cut 'Rojo Brillante' persimmon. A previous study by our group showed that the application of a similar coating also reduced pathogen populations in artificially inoculated persimmon when compared to other antimicrobial food additives.[10] In that experiment, however, the impact upon the population of *L. monocytogenes* was greater, whereas in the present study, the coating was more effective on the reduction of *E. coli* and *S. enteritidis* populations for both packaging conditions (active MAP and air). The effectiveness of NI in inhibiting the growth of Gram-positive bacteria is well-known. For example, NI inhibited the growth of *L. monocytogenes* in processed mangoes or melons.[26,27] However, some works have described a resistance of *L. monocytogenes* to NI, which was explained by a mutation of the bacteria that caused changes in the fatty acid composition of the cell membrane hindering NI insertion into the membrane.[28-30] Nevertheless, the effect observed in our study could be due to other factors, since the population of *L. monocytogenes* in coated samples packed in MAP conditions was significantly reduced after 8 days of storage at low temperature.

On the other hand, in the absence of other preservation methods, NI does not inhibit Gram negative bacteria or yeasts and moulds.[22] Therefore, NI is often used in combination with other preservation methods such as lowering the pH, addition of high salt concentrations, or the use of other chelating agents to achieve a bactericidal effect toward both Gram-positive and Gram-negative bacteria. In these cases, the effect of NI on Gram-negative bacteria is achieved so long as the outer bacteria cell membrane, which acts as a shield, is destroyed. For instance, treatments with NI and some chelators such as EDTA or certain acids, reduced the population of Gram-negative bacteria.[31,32] Therefore, the low pH of the pectin-based edible coating (pH 2.30), due to the addition of CA, might have enhanced the effect of NI against the different food-borne pathogens tested.

Colour and polyphenol oxidase (PPO) activity of fresh-cut persimmon

Figure 4 shows the effect of the pectin-based edible coating and packaging conditions on hue angle and a* values of fresh-cut persimmon during storage at 5 ºC. Coated persimmon slices maintained lower a* and higher hue values than uncoated samples during the 9 days of storage, which indicates the positive effect of the pectin coating to control enzymatic browning of the samples. In uncoated samples, the use of MAP helped maintaining lower a* and higher hue values of persimmon slices than air conditions (Control). The application of MAP to the coated samples further reduced initial enzymatic browning compared to those samples stored in air conditions, as reflected by a decrease in a* and an increase in hue values after 2 days of storage at 5 ºC. However, the differences were reduced as storage time at 5 ºC increased. In a previous work, the use of active MAP (5 kPa O_2) significantly reduced the enzymatic browning of untreated persimmon slices compared to those packed under passive MAP conditions, whereas packaging conditions did not affect the colour parameters of antioxidant-treated samples (unpublished data).

Several works have reported the effectiveness of polysaccharide coatings to control 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 the antibrowning substance incorporated and the edible coating selected. In preliminary work conducted by our group, pectin and hydroxypropyl methylcellulose-based edible coatings containing CA and $CaCl_2$ proved more effective to extend the commercial shelf life of fresh-cut persimmon than soy protein isolate- or whey protein isolate-based coatings containing the same antibrowning agents (unpublished data). Other research works have also reported the positive effect of the incorporation of antioxidants to polysaccharide edible coatings to control enzymatic browning of fresh-cut fruits. Thus, pectin-, gellan-, and alginate coatings containing N-acetylcysteine and glutathione as antioxidants were effective in avoiding browning of fresh-cut pears [8] and the incorporation of ascorbic acid into an alginate-based coating contributed to colour retention of fresh-cut mango.[33]

On the other hand, some attempts have also been focused on extending the shelf life of fresh-cut commodities by combining both edible coatings and MAP. For example, the combination of soy protein coatings with antibrowning agents and MAP has been evaluated by our group on fresh-cut artichoke, eggplant, and persimmon.[13,34,35] MAP conditions included passive MAP, active conventional MAP (5 kPa O_2 + 15 kPa CO_2), and high O_2 MAP (>50 kPa, balanced with N_2) and they were compared to atmospheric conditions as control. Coating application in atmospheric packaging conditions provided the best and cheapest approach for extending the shelf life of fresh-cut eggplants and artichokes.[34,35] On the contrary, the combination of soy protein coating with active conventional MAP showed a synergic effect in controlling tissue browning of fresh-cut 'Rojo Brillante' persimmon.[13]

The effect of the pectin-based coating to control browning correlated with a lower PPO activity in coated persimmons compared to uncoated ones (Fig. 5), whereas the use of active MAP slightly affected the enzyme activity compared to atmospheric conditions. The effect of the coating on PPO activity can be attributed to the effect of the antibrowning ingredients (citric acid and $CaCl_2$). Carboxylic acids such as citric acid have been reported to exhibit a double inhibitory effect by chelating copper, a key component of the PPO activity, and reducing the pH below that necessary for optimal PPO activity.[36] In persimmon, the optimum PPO activity has been reported to fall within the pH range of 5.5-7.5, depending on the substrate.[37,38] Thus, the low pH of the pectin-based coating (pH 2.3) might be the main factor that contributed to reduce the PPO activity. On the other hand, although low O_2 MAP has been reported to affect the PPO activity of fresh-cut commodities, its effect in this work was only observed for uncoated samples after 7 days of storage.

Firmness of fresh-cut persimmon

Firmness of fresh-cut persimmon decreased from an initial value of 33.3±1.7 N to values close to 15 N after 9 days of storage, except for uncoated samples stored in active MAP, which maintained firmness values above 20 N (Fig. 6). It is well known the effect of MAP with high CO_2 and low O_2 on reducing softening during postharvest storage of fruits and vegetables, which has been attributed to the reduction of either the activity of cell-wall-degrading enzymes or the metabolic activity of the product.[39] For example, the application of 4 kPa O_2 + 5 kPa CO_2 delayed firmness loss in fresh-cut pineapple [40], and similar atmospheric conditions positively influenced firmness in fresh-cut apples compared to air conditions.[41] In this work, the application of active MAP only helped to retain firmness of uncoated samples, whereas coated samples were not benefited by MAP. The effectiveness of edible coatings on preventing firmness depends on many factors, such as coating composition, commodity or stage of maturity. Thus, for example, Shon and Haque [42] reported no effect of calcium caseinate or whey protein-based coatings on the firmness of fresh-cut apples and potatoes, whereas Lee et al. [43] observed that the application of whey protein concentrate-based coatings to minimally processed apples reduced losses of firmness compared to uncoated samples. On the contrary, a carrageenan-based coating did not improve apple firmness and the addition of citric acid induced tissue softening. Previous work with fresh-cut 'Rojo Brillante' persimmon showed that acidic antibrowning agents such as citric or ascorbic acid, although effective in preventing enzymatic browning, led to major tissue softening and their combination with $CaCl_2$ was required to prevent excessive softening and maintain firmness within the same range than the control samples.[44]

Sensory quality of fresh-cut persimmon

The application of the pectin-based edible coating and active MAP maintained the visual quality of fresh-cut 'Rojo Brillante' persimmon within the limit of marketability during the 9 days of storage at 5 ºC, whereas uncoated samples packaged in air conditions were scored below this limit after 2 days of storage (Fig. 7). Overall, the judges scored the samples subjected to the combination of coated and active MAP with a value of 7 (very good) during the 9 days of storage, whereas samples subjected to each technology separately were scored as 5 (limit of marketability) by day 5, which indicates the synergic effect of both treatments to extend the shelf-life of 'Rojo Brillante' persimmon during storage at 5 ºC.

The combination of coating and MAP induced a slight off-flavour (scored as 2) to persimmon slices after 7 days of storage (Table 1). Despite of this, at the end of the 9-day storage period all the persimmon slices were evaluated with an overall flavour within the limit of acceptability (5.6-7.0 range). Coated persimmon slices were evaluated as less firm than uncoated samples at the end of storage, which confirmed the results from the instrumental texture analysis (Table 1; Fig. 6). However, the judges were not able to differentiate sensory firmness in uncoated samples subjected to different packaging conditions.

CONCLUSION

The application of a pectin-based coating formulated with antibrowning agents and NI as antimicrobial significantly extended the shelf-life of 'Rojo Brillante' persimmon slices by controlling enzymatic browning and reducing the growth of total aerobic mesophilic bacteria during storage at 5 ºC. Overall, the combination of the edible coating and active MAP (5 kPa O_2) proved to be the most effective treatment to maintain the visual quality of persimmon slices, being evaluated as very good at the end of the 9-day storage period, while the overall flavour fell within the limit of acceptability. The antimicrobial pectin coating also effectively stunted the growth of *E. coli*, *S. enteritidis* and *L. monocytogenes* in artificially inoculated fresh-cut persimmon.

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Table 1. Sensory quality of uncoated or pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) during 9 days at 5 ºC.

	Treatment	Days of storage			
		2	5	7	9
Off-flavour	Coating - MAP	1.6 ± 0.3a	1.6 ± 0.2a	2.1 ± 0.3a	2.1 ± 0.3a
	MAP	1.2 ± 0.1a	1.5 ± 0.3a	1.1 ± 0.1b	1.1 ± 0.1b
	Coating	1.6 ± 0.2a	1.5 ± 0.2a	1.3 ± 0.1b	1.3 ± 0.1b
	Control	1.5 ± 0.3a	1.2 ± 0.1a	1.3 ± 0.2b	1.3 ± 0.2b
Flavour	Coating - MAP	6.5 ± 0.4a	6.3 ± 0.4a	5.0 ± 0.3b	5.6 ± 0.5b
	MAP	6.6 ± 0.4a	7.1 ± 0.3a	6.9 ± 0.2a	6.9 ± 0.3a
	Coating	6.4 ± 0.4a	6.6 ± 0.3a	6.5 ± 0.2a	6.0 ± 0.4ab
	Control	6.9 ± 0.4a	6.7 ± 0.4a	6.7 ± 0.4a	7.0 ± 0.3a
Firmness	Coating - MAP	2.9 ± 0.3b	2.8 ± 0.2b	2.6 ± 0.2b	2.7 ± 0.2b
	MAP	3.8 ± 0.2a	3.8 ± 0.2a	3.4 ± 0.2a	3.3 ± 0.3a
	Coating	3.2 ± 0.2ab	3.5 ± 0.2ab	3.2 ± 0.2ab	2.5 ± 0.2b
	Control	3.1 ± 0.3ab	4.0 ± 0.2a	3.6 ± 0.2a	3.3 ± 0.2a

Sensory quality of persimmon fruit at processing: Off-flavour = 1.0 ± 0.0; Flavour = 7.8 ± 0.2; Firmness = 4.1 ± 0.2.
For each paramenter and storage time, different letters indicate significant differences among treatments by the least significant difference (LSD) test ($P \leq 0.05$).
Data are mean ± standard error.

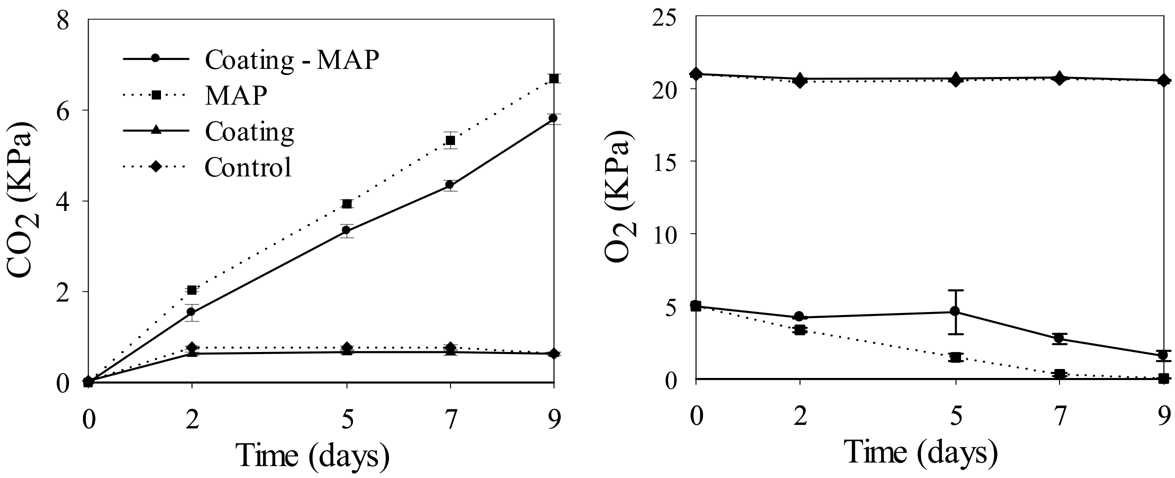

Figure 1. Concentration of CO_2 and O_2 in the headspace gas composition of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Vertical bars represent the standard error.

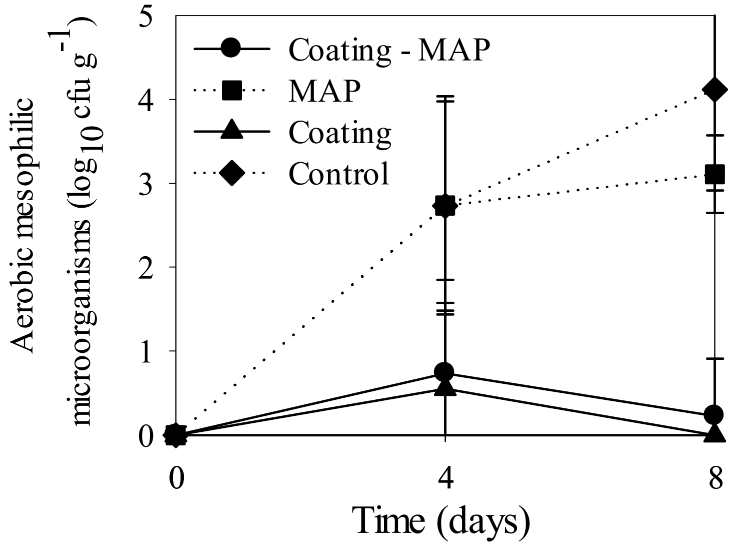

Figure 2. Growth of aerobic mesophilic bacteria in uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 8 days at 5ºC. Vertical bars show the standard error.

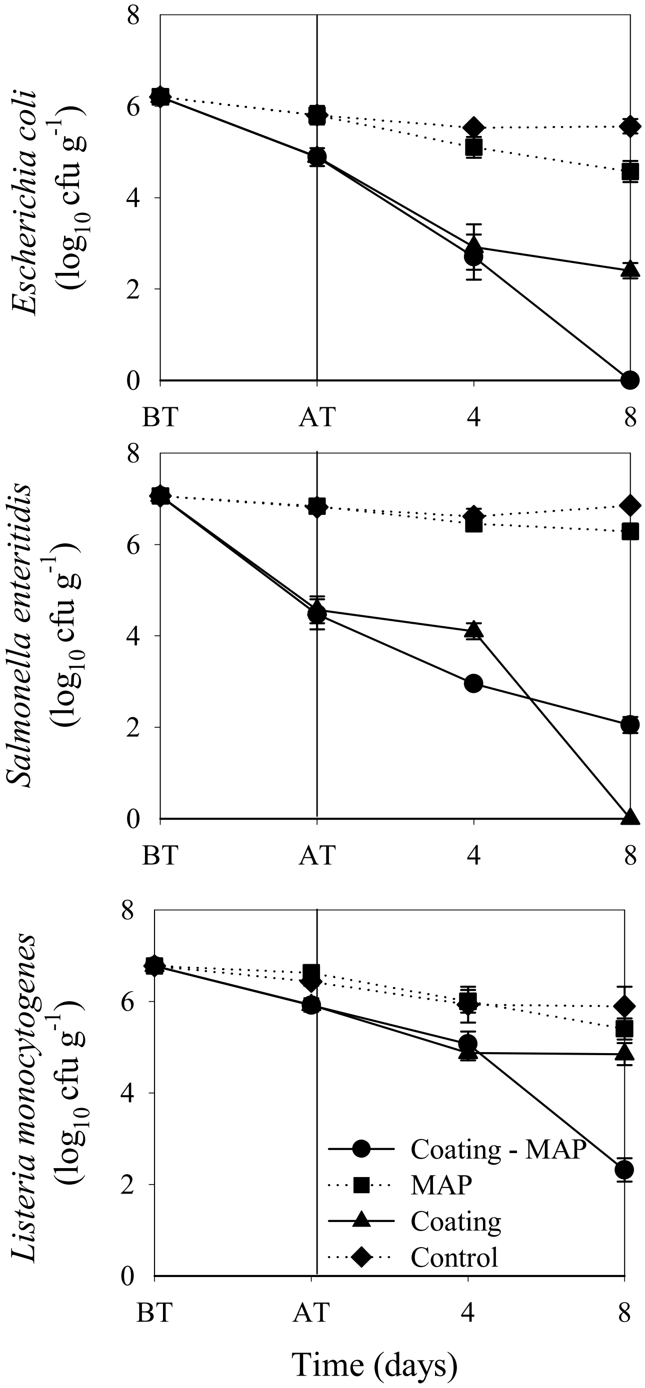

Figure 3. Populations of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* in uncoated and pectin-based coated minimally processed 'Rojo Brillante' persimmon plugs before (BT) and after treatment (AT) (coating or water dips), and after 4 and 8 days of storage at 5 ºC in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2). Vertical bars show the standard error.

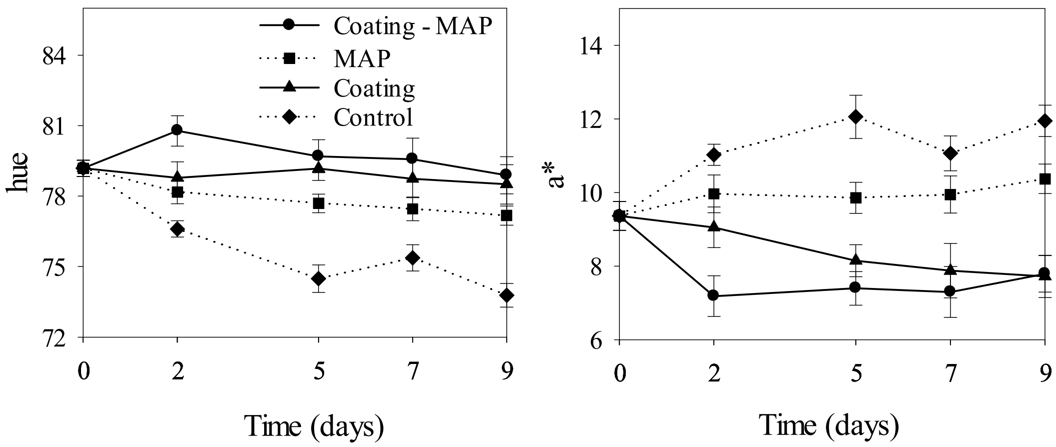

Figure 4. Flesh color hue and *a** values of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Vertical bars show the standard error.

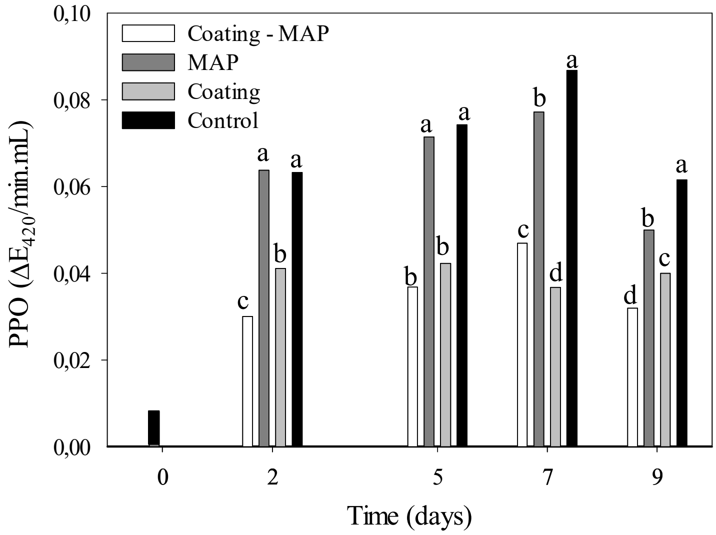

Figure 5. Polyphenol oxidase (PPO) of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. For each storage time, bars with a different letter are significantly different at the 95% level.

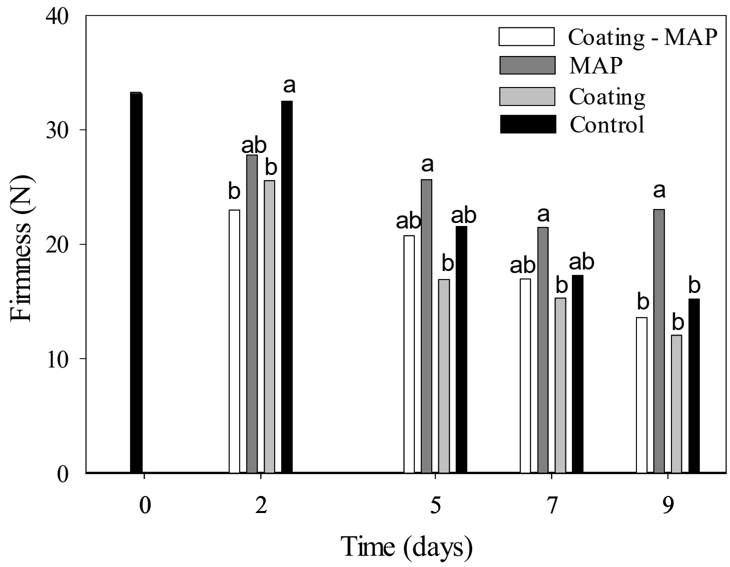

Figure 6. Firmness of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. For each storage time, bars with a different letter are significantly different at the 95% level.

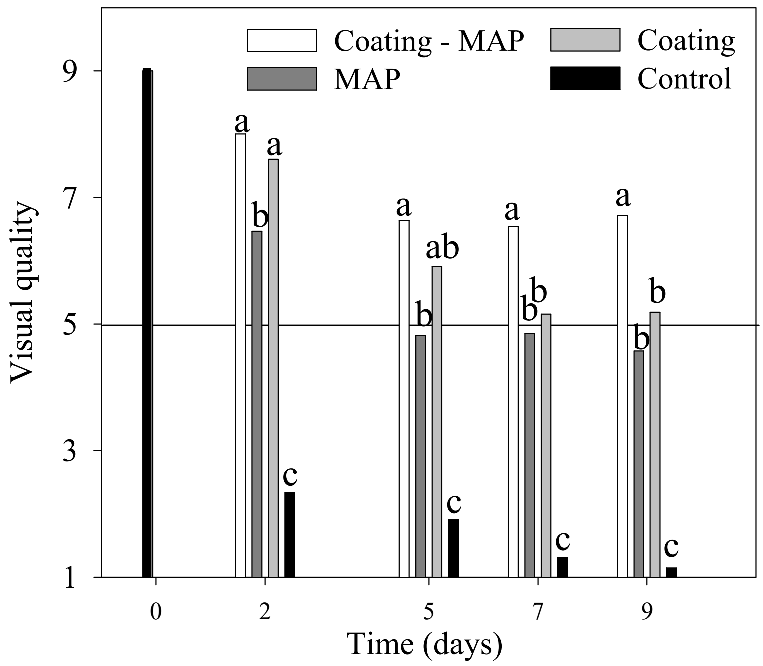

Figure 7. Visual quality of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmons packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Visual scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible. For each storage time, bars with a different letter are significantly different at the 95% level.