UNIVERSIDAD POLITÉCNICA DE VALENCIA
Instituto Universitario de Ingeniería de Alimentos para el Desarrollo

EFFECT OF PROCESSING ON THE PHYSICOCHEMICAL, SENSORY, NUTRITIONAL AND MICROBIOLOGICAL QUALITY OF FRESH-CUT 'ROJO BRILLANTE' PERSIMMON

EFECTO DEL PROCESADO EN LA CALIDAD FISICOQUÍMICA, SENSORIAL, NUTRICIONAL Y MICROBIOLÓGICA DE CAQUI 'ROJO BRILLANTE' FRESCO CORTADO

TESIS DOCTORAL

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Dra. María Bernardita Pérez Gago, Investigadora del Centro de Tecnología Poscosecha del Instituto Valenciano de Investigaciones Agrarias,

CERTIFICA: Que la memoria titulada "Efecto del procesado en la calidad físicoquímica, sensorial, nutricional y microbiológica de caqui 'Rojo Brillante' fresco coortado", que, para aspirar al grado de Doctor en Ciencia, Tecnología y Gestión Alimentaria presenta Dª. Elena Sanchís Soler, realizada bajo mi dirección en el Centro de Tecnología Poscosecha del Instituto Valenciano de Investigaciones Agrarias, cumple las condiciones adecuadas para su aceptación como Tesis Doctoral, por lo que

AUTORIZA: Al interesado a su presentación en el Instituto Universitario de Ingeniería de los Alimentos para el Desarollo de la Universidad Politécnica de Valencia.

Y para que conste a los efectos oportunos, presento la referida memoria, firmando el presente certificado en Valencia a 14 de Octubre de 2015.

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Nuestra recompensa se encuentra en el esfuerzo y no en el resultado. Un esfuerzo total es una victoria completa.

Mahatma Gandhi

Als meus pares i el meu germà. Per donar-m'ho tot en esta vida. Sense vosaltres mai haguera arribat fins ací.

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El caqui persimmon (*Diospyros kaki* L.) 'Rojo Brillante' es un cultivar astringente que presenta unas propiedades organolépticas y nutricionales excelentes. En la última década, su cultivo en el área mediterránea de España se ha incrementado de manera exponencial con el desarrollo de la tecnología que permite eliminar la astringencia, manteniendo la firmeza del mismo. Esta nueva forma de presentación, aporta numerosas ventajas, entre la que se incluye la posibilidad de ser comercializado como fruta fresca cortada. Sin embargo, el éxito comercial del producto está limitado por el pardeamiento enzimático, la pérdida de firmeza y al crecimiento microbiano.

En este contexto, el objetivo de la Tesis ha sido el desarrollo de caqui 'Rojo Brillante' fresco cortado mediante un enfoque que integra el estudio de las características del producto en el momento del procesado y de distintas tecnologías que mantengan la calidad físico-química, sensorial, nutricional y microbiológica del producto durante un periodo que permita su comercialización.

En primer lugar, se evaluó el efecto del estado de madurez (MS) en el momento de recolección, el tiempo de almacenamiento a 15 ºC antes del procesado y la aplicación de diferentes antioxidantes en el pardeamiento enzimático y la calidad sensorial y nutricional del caqui 'Rojo Brillante' cortado y almacenado a 5 ºC. La aplicación de 10 g L^{-1} de ácido ascórbico (AA) ó 10 g L^{-1} ácido cítrico (CA) controló el pardeamiento enzimático y mantuvo la calidad visual del caqui por encima del límite de comercialización entre 6 y 8 días de almacenamiento a 5 ºC, dependiendo del MS. Sin embrago, la aplicación de estos antioxidantes redujo de manera significativa la firmeza del fruto respecto al control. La combinación de estos antioxidantes con 10 g L^{-1} de $CaCl_2$ permitió mantener la firmeza en el mismo rango que las muestras control.

En un trabajo posterior, la aplicación de 1-metilciclopropeno (1-MCP) permitió procesar caqui almacenado 45 días a 1 ºC con una buena firmeza comercial y el tratamiento antioxidante (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) consiguió alcanzar un límite de comercialización del producto de 9 días a 5 ºC.

La evaluación de distintas atmósferas controladas en combinación con tratamientos antioxidantes (AA o CA), como paso previo al envasado en

atmósfera modificada (MAP) del caqui, mostró como más efectiva en el control del pardeamiento enzimático la atmósfera compuesta por 5 kPa O_2 (balance N_2). Esta atmósfera mantuvo la calidad visual del caqui cortado dentro del límite de comercialización durante 7-9 días a 5 ºC. Por el contrario, la aplicación de altas concentraciones de CO_2 (10 ó 20 kPa) dio lugar a un pardeamiento en ciertas zonas de la pulpa que se conoce como 'internal flesh browning'. Estudios posteriores confirmaron el efecto beneficioso del envasado de caqui cortado y tratado con solución antioxidante (CA-$CaCl_2$) en una MAP activa de 5 kPa O_2 en la calidad visual del fruto frente a la aplicación de una MAP pasiva.

El desarrollo de recubrimientos comestibles con capacidad antioxidante se realizó mediante la incorporación de antioxidantes (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) a formulaciones a base de proteína de suero lácteo (WPI), proteína de soja (SPI), hidroxipropilmetilcelulosa (HPMC) y pectina. Todos los recubrimientos fueron efectivos controlando el pardeamiento enzimático del caqui cortado, siendo las muestras recubiertas con HPMC y pectina las mejor evaluadas visualmente.

En general, el procesado, la aplicación de antioxidantes, el envasado en atmósferas controladas y los distintos recubrimientos comestibles estudiados, si bien no mostraron un efecto claro en los parámetros de calidad nutricional evaluados, no tuvieron un efecto negativo en los mismos. Por otra parte, los frutos cosechados a final de campaña tuvieron mayor actividad antioxidante y contenido en carotenoides.

Los recubrimientos comestibles con actividad antimicrobiana fueron preparados a partir de pectina, antioxidantes (CA-$CaCl_2$) y diferentes agentes antimicrobianos (2 o 4 g Kg^{-1} sorbato potásico (PS), 4 g Kg^{-1} benzoato sódico (SB), o 500 UI mL^{-1} nisina (NI)). Todos ellos fueron efectivos controlando el pardeamiento enzimático de caqui cortado. El recubrimiento con NI fue el más efectivo inhibiendo completamente el crecimiento de bacterias aeróbias mesófilas tras 4 días de almacenamiento a 5 ºC y también inhibió el crecimiento de *Escherichia coli*, *Salmonella enteritidis* y *Listeria monocytogenes* en caqui cortado e inoculado artificialmente. La combinación de este recubrimiento con el envasado en MAP activa (5 kPa O_2) mejoró la calidad visual del caqui cortado, además de inhibir el crecimiento microbiano, permitiendo superar un periodo de comercialización superior a 9 días a 5 ºC.

El caqui persimmon (*Diospyros kaki* L.) 'Rojo Brillante' és un cultiu astringent que presenta unes propietats organolèptiques i nutricionals excel·lents. En la última dècada, el seu cultiu en l'àrea mediterrània d'Espanya s'ha incrementat de manera exponencial amb el desenvolupament de la tecnologia que permet eliminar l'astringència, mantenint la fermesa del mateix. Esta nova forma de presentació, aporta un gran nombre d'avantatges, entre els quals s'inclou la possibilitat de comercialitzar-lo com fruita fresca processada. No obstant, l'èxit comercial del producte està limitat per pardetjament enzimàtic, la pèrdua de fermesa i el creixement microbià.

L'objectiu de la Tesis ha estat en el desenvolupament de caqui 'Rojo Brillante' tallat en fresc mitjançant un enfocament que integra l'estudi de les característiques del producte en el moment del processat i de diferents tecnologies en el manteniment de la qualitat físico-química, sensorial, nutricional i microbiològica del producte durant un període que permeta la seua comercialització.

En primer lloc, es va avaluar l'efecte de l'estat de maduresa (MS) en el moment de recol·lecció, el temps d'emmagatzemament a 15ºC abans del processat i l'aplicació de diferents tractaments antioxidants en el pardetjament enzimàtic i la qualitat sensorial i nutricional del caqui 'Rojo Brillante' tallat i emmagatzemat a 5 ºC. L'aplicació de 10 g L^{-1} d'àcid ascòrbic (AA) o 10 g L^{-1} d'àcid cítric (CA) va controlar el pardetjament enzimàtic i va mantenir la qualitat visual del caqui per damunt del límit de comercialització entre 6-8 dies d'emmagatzemament a 5 ºC, depenent del MS. No obstant, l'aplicació d'antioxidants va reduir de manera significativa la fermesa del fruit comparat amb el control. La combinació d'aquestos antioxidants amb 10 g L^{-1} de $CaCl_2$ va permetre mantenir la fermesa en el mateix rang que les mostres control.

En un treball posterior, l'aplicació de 1-metilciclopropeno (1-MCP) va permetre processar caqui emmagatzemat 45 dies a 1 ºC amb una bona fermesa comercial i a més, el tractament antioxidant (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) va aconseguir un límit de comercialització del producte tallat de 9 dies a 5 ºC.

L'avaluació de diferents atmosferes controlades en combinació amb tractaments antioxidants (AA o CA), com a pas previ a l'envasament en

atmosfera modificada (MAP) del caqui 'Rojo Brillante, va mostrar com a més efectiva en el control del pardetjament enzimàtic l'atmosfera composta per 5 kPa O_2 (balanç N_2). Aquesta atmosfera va mantenir la qualitat visual del caqui tallat dins del límit de comercialització durant 7-9 dies a 5 ºC. Per contra, l'aplicació d'altes concentracions de CO_2 (10 ó 20 kPa) va donar lloc a un pardetjament en certes zones de la polpa, el qual és conegut com 'internal flesh browning'. Estudis posteriors van confirmar l'efecte beneficiós de l'envasament de caqui tallat i tractat amb solució antioxidant (CA-$CaCl_2$) en una MAP activa de 5 kPa O_2 millorant la qualitat visual de la fruita front a l'aplicació de una MAP passiva.

El desenvolupament de recobriments comestibles amb capacitat antioxidant es va realitzar mitjançant la incorporació d'antioxidants (CA-$CaCl_2$) en formulacions a base de proteïna de sèrum làctic (WPI), proteïna de soia (SPI), hidroxipropilmetilcel-lulosa (HPMC) i pectina. Tots els recobriments van ser efectius controlant el pardetjament enzimàtic del caqui tallat. No obstant, les mostres recobertes amb HPMC i pectina van ser millor avaluades visualment que la resta de tractaments.

En general, el processat, l'aplicació d'antioxidants, l'envasament en atmosferes controlades i els distints recobriments comestibles estudiats, si bé no van mostrar un efecte clar en els paràmetres de la qualitat nutricional avaluats, no van tindre un efecte negatiu en els mateixos. Per altra banda, els fruits recol·lectats a final de temporada van tenir major activitat antioxidant i contingut en carotenoides.

Els recobriments comestibles amb activitat antimicrobiana van ser formulats a partir de pectina, antioxidants i diferents agents antimicrobians (2 o 4 g Kg^{-1} sorbat potàssic (PS), 4 g Kg^{-1} benzoat sòdic (SB), o 500 UI mL^{-1} nisina (NI)). Tots ells van ser efectius controlant el pardetjament enzimàtic del caqui tallat. El recobriment amb NI va ser el més efectiu inhibint completament el creixement de bactèries aeròbiques mesòfiles després de 4 dies d'emmagatzemament a 5 ºC i també va inhibir el creixement de *Escherichia coli, Salmonella enteritidis* y *Listeria monocytogenes* en caqui tallat e inoculat artificialment. La combinació d'aquest recobriment amb l'envasament en MAP activa (5 kPa O_2) va millorar la qualitat visual del caqui, a més d'inhibir el creixement microbià, permetent superar un període de comercialització superior a 9 dies a 5 ºC.

Persimmon (*Diospyros kaki* L.) 'Rojo Brillante' is an astringent variety characterised by good growing conditions, excellent colour, size, sensory characteristics and good nutritional properties. In the last decade, its production has grown substantially in Spain given the application of high levels of CO_2 to remove astringency while firmness is preserved. This technology has also increased its potential as a fresh-cut commodity. However, physical damage during processing result in degradation of the colour and firmness of the product and a higher susceptibility to microbial spoilage that significantly reduces the fruit´s shelf life.

The objective of the present thesis was to develop optimum procedures for processing and marketing 'Rojo Brillante' persimmon into a fresh-cut product with the maximum shelf life and best physicochemical, nutritional, sensory and microbiological quality.

Firstly, the objective was to evaluate the effect of the maturity stage (MS) at harvest, storage time at 15 ºC before processing, and the application of different antioxidant treatments on enzymatic browning, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. Concentrations of 10 g L^{-1} ascorbic acid (AA) or 10 g L^{-1} citric acid (CA) controlled tissue browning and maintained the visual quality of fresh-cut persimmon above the limit of marketability for 6-8 storage days at 5 ºC, depending on the MS. However, these acidic solutions reduced fruit firmness as compared to control samples. Further studies showed that the combination of these antioxidants with 10 g L^{-1} $CaCl_2$ maintained firmness of the persimmon slices within the same range as the control samples.

In another work, the application of 1-methylcyclopropene (1-MCP) allowed to process fruits after 45 days of storage at 1 ºC with commercial firmness and the antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) extended the limit of marketability up to 9 days of storage at 5 ºC.

Different controlled atmosphere conditions in combination with AA or CA dips were also evaluated as a first step to select optimum O_2 and CO_2 concentrations for modified atmosphere packaging (MAP) of fresh-cut 'Rojo Brillante' persimmons. Overall, the combination of antioxidant dips and a controlled atmosphere composed of 5 kPa O_2 (balance N_2) was proved to be the most effective combination to control enzymatic browning. This atmosphere maintained the visual quality of persimmon slices within the

limit of marketability during 7- 9 days at 5 ºC. On the contrary, high CO_2 concentrations (10 or 20 kPa) induced darkening in some tissue areas, associated with a flesh disorder known as 'internal flesh browning'. Later studies confirmed the beneficial effect of an active MAP in 5 kPa O_2 compared to passive MAP to improve the visual quality of fresh-cut 'Rojo Brillante' persimmon, showing a synergic effect with the antioxidant dip (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$).

Antioxidant edible coatings were prepared from whey protein isolate (WPI), soy protein isolate (SPI), hydroxylpropyl methylcellulose (HPMC) and apple pectin as the polymeric matrix. All edible coatings were amended with the antioxidant combination selected (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$). All the edible coatings tested proved effective to control enzymatic browning of persimmon slices. However, the samples treated with the HPMC- and pectin- based coatings were scored with a better visual quality that the rest of the treatments.

In general, free radical scavenging activity and total carotenoid content increased in late-season persimmons; whereas, processing (cutting and storage at 5 ºC), antioxidant dips, controlled atmosphere storage or edible coatings had no clear effect on nutritional quality (vitamin C, free radical scavenging activity, total phenolic content, and carotenoids) of fresh-cut persimmons.

Antimicrobial edible coatings were prepared from the optimised apple pectin-based edible coating by incorporating different antimicrobial agents (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}). All the edible coatings tested were effective to control enzymatic browning of fresh-cut persimmon. The combination of antioxidants with NI completely inhibited the growth of mesophilic aerobics after 4 days at 5ºC and also effectively stunted the growth of *Escherichia coli, Salmonella enteritidis* and *Listeria monocytogenes* in artificially inoculated fresh-cut persimmons. Combination of active MAP (5kPa O_2) and the selected pectin-based edible coating improved the visual quality of coated persimmon slices and inhibited microbial growth, which extended the shelf-life for more than 9 days of storage at 5 ºC.

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INTRODUCTION

1. General overview of persimmon fruit

Persimmons are the edible fruit of a number of species of trees in the genus *Diospyros kaki* L. in the family Ebenaceae. This genus includes hundreds of different persimmon cultivars that differ in shape, size, colour and level of astringency upon harvest. According to the level of astringency upon harvest, persimmon cultivars are classified into non-astringent (NA) and astringent (A) persimmons, which differ on the concentration of the soluble tannins present in fruit flesh. In both categories, there are cultivars in which fruit astringency is influenced by pollination (pollination variant, PV) and cultivars whose fruits are not affected by pollination (pollination constant, PC). Accordingly, Belini (1982) classified persimmon fruits into four groups: 1) the PCNA group, which is not astringent and it can be with or without seeds, and persimmons can be eaten at harvest when they are firm; 2) the PVNA group, which includes not astringent fruits at harvest if they have seeds and astringent fruits when firm if they have been not pollinated; 3) the PCA group, which is always astringent when firm; 4) the PVA group, which is also astringent if pollinated, and is not astringent only around seeds where they have dark tannin spots (Figure 1).

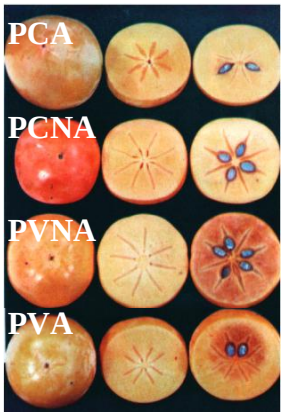

Figure 1. Persimmon classification according to astringency

Persimmon fruit originated from China and it was extended to other warm regions of the world. In the Mediterranean region, persimmon fruit

was introduced by the end of the 19th century, first as an ornamental tree and it was later spread to coexist with other crops such as citrus, pomegranate, fig and olive trees. For many decades, the fruit was grown as isolated trees, in gardens, family orchards or in small plantations destined to local consumption (Llácer and Badenes, 2002). Nowadays, persimmon fruit represents an important commercial crop worldwide.

In the last decade, persimmon worldwide production has been duplicated to reach 4,468,960 tones in 2012 (Table 1) (FAOSFAT, 2014). Among countries, China is the main producer with 75% of the total, followed by Republic of Korea, Japan and Brazil. In the Mediterranean area, the main producers in order of importance are Spain, Azerbaijan, Italy, Uzbekistan and Israel, being Spain the country with the largest and most rapid expansion.

Table 1. Evolution of persimmon production (tonnes) from the main producing countries in the last years

	2000	2002	2004	2006	2008	2010	2012
Australia	759	866	881	700	720	666	660
Brazil	63,300	141,364	162,288	168,274	173,297	167,215	158,241
China	1,615,800	1,775,340	2,034,390	2,346,740	2,744,890	3,046,400	3,386,000
Italy	42,450	54,170	57,110	53,100	50,000	48,969	47,000
Japan	278,800	269,300	232,500	232,700	266,600	189,400	253,800
Mexico	247	192	275	287	362	363	244
New Zealand	1,352	2,900	3,000	2,428	2,900	2,620	2,350
R, of Korea	287,847	281,143	299,046	352,822	430,521	390,630	401,049
Total world	2,391,660	2,684,090	2,897,820	3,335,700	3,880,340	4,058,290	4,468,960

Source: FAOSFAT (2014).

Nowadays, the persimmon production in Spain has exceeded 150,000 tonnes compared to a production around 15,000 tonnes in year 2000, being considered an alternative to other Mediterranean crops such as citrus (Perucho, 2015). The production focuses on variety 'Rojo Brillante' (94% of the production), which is mainly produced in the 'Ribera del Xúquer' area in Valencia province, followed by variety 'Triumph' (6% of the production), produced mainly in Andalucia.

Persimmon 'Rojo Brillante' is an astringent variety at harvest (PCA), characterised by good growing conditions, excellent colour, size, sensory characteristics and good nutritional properties (Salvador et al., 2007). Due to its astringency, this cultivar was traditionally consumed over-

ripened as soft persimmon and its commercialisation only reached local markets, since the fruit badly support handling and transport. The optimisation of the technique to remove fruit astringency while firmness is preserved was a substantial improvement in fruit marketing and exporting and was the main reason for the rapid expansion of the cultivar. The great impact of 'firm persimmon' 'Rojo Brillante' in the economy of the 'Ribera del Xuquer' area led to the creation of the Council Regulator of the Denomination of Origin (CRDO) 'Kaki Ribera del Xúquer' in 1997 that guarantees the quality and origin of persimmon 'Rojo Brillante' from this area.

Astringency in persimmon fruits is due to the presence of tannins which form stable complexes with salivary proteins and cause them to precipitate or aggregate, which leaves a rough sensation in the mouth (Nakatsubo et al., 2002). The current method used to remove astringency and maintain firmness, involves holding the fruit in air-tight chambers for 24 h under 95–98% CO_2 at 20 ºC and 90% R.H. (Arnal and Del Río, 2003). The effectiveness of this method lies in the fact that it triggers anaerobic respiration in the fruit, which gives rise to an accumulation of acetaldehyde and then a reaction between this acetaldehyde and the soluble tannins that are responsible for the astringency (Salvador et al., 2007). The tannins then become insoluble, which are non-astringent (Taira et al., 1997).

Upon the optimisation of the technology that allows the commercialisation of firm persimmon 'Rojo Brillante', numerous studies have been focused on evaluating different postharvest treatments to preserve fruit quality during cold storage and to extend the fruit storage period to supply the markets according to the demand for its consumptions in fresh.

The harvest season of 'Rojo Brillante' cultivar takes place between October and December. After the astringency has been removed, fruit firmness is the main property taken into account in order to rate quality. At present values below 10 N following storage and marketing have been considered unsuitable to commercialise the fruit as firm (Arnal and Del Río, 2004). Nevertheless, the external colour of the persimmon is the property used as a non-destructive index for harvesting. In 'Rojo Brillante' fruit, the colour of the epidermis varies from green to a bright

red when the fruit is in the last stages of ripeness (Figure 2) (Salvador et al., 2007). The green colour of persimmon fruit in stages I-II and the significant degree of softening in stage VI limit in most of the cases the harvest period to stages III to V.

Figure 2. External appearance of 'Rojo Brillante' persimmon at six different maturity stages (Salvador et al., 2007)

Storage of persimmon fruits is a common way of managing supply and to also prolong the commercial period. Although the standard recommended storage temperature for persimmon fruits is 0 ºC (Crisosto, 2004), some cultivars such as 'Rojo Brillante' are sensitive to low temperatures, and they develop physiological disorders (chilling injury, CI) when exposed at temperatures below the optimal (Arnal and Del Río, 2004). In the cultivar 'Rojo Brillante', CI symptoms develop when the fruit is stored at temperatures below 11 ºC and they manifest as is major loss of firmness (Arnal and Del Río, 2004; Salvador et al., 2007). On the other hand, storage at 15 ºC, as recommended temperature to avoid CI, only extend the limit of storability to periods no longer than 20 days due to progressive softening (Besada et al., 2008).

Several research works have focused on the study of postharvest treatments to alleviate CI and to extend the storage period. These include heat treatments such as hot water and hot air, controlled and modified

atmosphere storage and the use of exogenous 1-Methylcyclopropene (1-MCP). Among them, 1-MCP, which is an inhibitor of ethylene action, has been very effective in alleviating chilling injury symptoms and maintaining firmness of persimmon 'Rojo Brillante' during cold storage (Salvador et al., 2004). Nowadays, its use has become a common practice in the packing houses to prolong the storage time and extend the campaign of this cultivar. Other technologies that have been studied are the application of hot air treatments (Arnal and Del Río, 2003), hot water treatments before cold storage (Besada et al., 2008), or controlled atmosphere storage in combination with 1-MCP (Besada et al., 2014). The response of these technologies, although effective to alleviate CI symptoms of 'Rojo Brillante' persimmons, was conditioned by the stage of maturity of the fruit and they are not applied at a commercial state.

Beside CI symptoms, the main postharvest disorder manifested by 'Rojo Brillante' persimmons is 'flesh browning'. Even though the cause of this disorder remains unknown, it has been related to pre-harvest nutritional deficiencies, mechanical injury during the postharvest period and the post-application of high CO_2 atmospheres to eliminate astringency (Zavrtanik et al., 1999; Besada et al., 2010). In recent studies, Novillo et al. (2014a, 2014b) reported that the incidence and severity of 'flesh browning' in 'Rojo Brillante' persimmons was greater the longer the CO_2 exposure time taken to remove astringency. This correlated with an accumulation of superoxide anion and H_2O_2, which suggests the implication of oxidative stress in this postharvest disorder.

Beyond the commercialisation of 'Rojo Brillante' persimmon as a fresh commodity with a sufficient commercial shelf life, the sector is also eager to look for alternatives to market this fruit in different formats that would expand the offer. In this sense, some works have been conducted to obtain persimmon jam (Castelló et al., 2011; Igual et al., 2011), juice (Hernández-Carrión et al., 2014; González et al., 2015) and dehydrated product (Igual et al., 2008). On the other hand, the technology to remove the astringency has also opened the possibility to prepare this cultivar as a fresh-cut commodity, which requires the study of the characteristics of the product at the time of processing and different technologies to maintain the physico-chemical, sensory, nutritional and microbiological quality of the product for a period to allow commercialisation

2. Minimally processed or fresh-cut produces

Minimally-processed or fresh-cut produces are ready-to-eat or ready-to-use fruits and vegetables that have been washed, cut, chopped and packaged into convenient forms that maintain their fresh nature (Cantwel, 1992). This trading form was developed in the 1980s to respond to the emerging consumer's demand for convenience, high quality and healthy food products with fresh like appearance. The demand for fresh-cut fruit and vegetables has continuously increased during the last years, being the convenience factor the main reason for the growth.

In Spain, although still far from the commercial figures of U.S.A and other European markets such as France, United Kingdom or Italy, the total sales of fresh-cut fruits and vegetables in 2014 amounted to 81,500 tonnes, which represented an increase of 4.9% over the previous year and an economic benefit above 300 million euros (FEPEX, 2015). Among them, vegetables (mainly bagged salads) constitute the main segment (≈ 97%) of fresh-cut products, with a market pretty much consolidated (Figure 3). Fresh-cut fruits, on the contrary, have grown at a lower rate due to their higher perishability. Nevertheless, it must be highlighted the significant increase in turnover of fresh-cut fruits, which in the last year increased by 4.7% in volume and 25.4% in value (Markets Magazine, 2015). Despite the observed increase of fresh-cut fruits in the market, the range of fresh-cut fruit is still very limited, focusing mainly in melon, watermelon, grapefruit, pineapple, pomegranate, grape, and their fruit mixes. Therefore, the development of new fruit products with good quality represents a challenge and a marketing opportunity for the food industry.

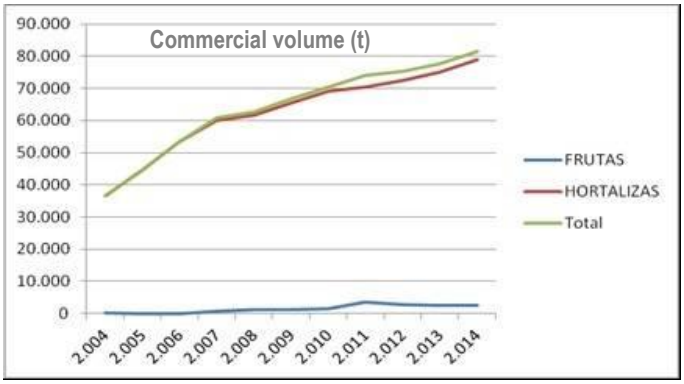

Figure 3. Commercial volume of fresh-cut fruits and vegetables in Spain. (Source: FEPEX 2015)

2.1. Effect of processing on quality of fresh-cut fruits

Quality is a combination of attributes, properties or characteristics that determine their value to the consumer. These include appearance, texture, flavour, nutritive value and safety of the product (Kader, 2002). Contrary to other food processing techniques such as drying, freezing or canning, fresh-cut processing does not extend the shelf-life of the product. In fact, processing of fresh-cut fruits generates physiological stresses in the tissue, leading to an increase in respiration rate, ethylene production, and metabolic changes, which result in degradation of the colour, flavour, and firmness of the product that significantly reduces the fruit's shelf life. In addition, cut fruit surfaces provide a favourable environment for microbial growth, due to increased moisture, sugars and analytes leaking from open cells.

Quality of fresh-cut fruits can be preserved to a certain extent by understanding the physiology of the fresh-cut fruit or vegetable and applying different techniques. Such techniques would be to choose the proper cultivar and ripeness stage, to use adequate sanitation procedures and processing techniques, to maintain low temperature from harvest to retail (cold chain), and to apply physical and chemical treatments such as modified atmosphere packaging (MAP) and/or antioxidant dips and edible coatings (Dea et al., 2012).

2.2. Physiological effects of cutting on tissues

The effects of processing on tissue metabolism can be observed very rapidly, often within minutes to a few hours after cutting (Toivonen and Brummell, 2008). One of the most common responses to wounding in plant tissue is an increase in respiration rate and ethylene production. In general, high respiration rates are directly associated with a rapid increase in the tissue metabolism and consequently with accelerated loss of acids, sugars, and other components (Cantwell and Suslow, 2002). Physiological changes induced by elevated ethylene concentration include increased cell permeability, loss of compartmentation, increased senescence and respiratory activity, as well as enzymatic activity (Hyodo et al., 1983).

The response of wounding to ethylene and respiration rate varies depending on the type of commodity, cultivar, ripeness stage, degree of injury, and storage temperature (Dea et al., 2012). Thus for examples, mango cubes and pear slices did not show an increase in ethylene production in response to cutting (Chantanawarangoon, 2000; Gorny et al., 2000), whereas other fruits such as kiwifruit (Agar et al., 1999), apple (Hu et al., 2007), papaya (Paull and Chen, 1997), strawberry (Rosen and Kader, 1989), and tomato (Artés et al., 1999) did. Similarly, a wide range of fresh-cut products shows significant increases in respiration, and generally, this effect is only seen when the cut product is stored at high temperatures, although some exceptions can be found (Toivonen and DeEll, 2002).

Another consequence of fresh-cut fruit processing is cell disruption, which allows previously compartmentalized endogenous enzymes to come into contact with their substrates, leading to undesiderable reactions such as enzymatic browning and accelerated softening due to enzymatic hydrolysis of the cell wall pectin substances. Moreover, peeling and removal of protective tissues lead to changes in gas diffusion, accelerates water loss, and favours microbial contamination.

Enzymatic browning is one of the most limiting factors on the shelf-life of fresh-cut products. Enzymatic browning is a complex process that can be subdivided in two parts. The first part is mediated by polyphenol oxidase (PPO, a copper-containing enzyme), resulting in the formation of

o-quinones (slightly colored), which through non enzymatic reactions, lead to the formation of complex brown pigments. *o*-Quinones are highly reactive and can rapidly undergo oxidation and polymerization. Usually, brown pigments are formed, but in addition, reddish-brown, blue-grey and even black discolorations can be produced on some bruised plant tissues (Garcia and Barret, 2002). The most important factors to determine the enzymatic browning are the concentration of PPO and phenolic compounds, pH, temperature and the oxygen availability in the tissue. In most cases, the optimum pH range for PPO activity is between pH 4 and 7 (Laurila et al., 1998).

Softening and loss of tissue firmness is a quality defect that compromises the shelf life of many fresh-cut fruits. Many of the textural changes occurring on fresh-cut fruit are a continuation of the normal ripening events that lead to softening and the cutting process accelerates softening (Toivonen and Brummell, 2008). In whole fruit, cell walls undergo a natural degradation during fruit ripening, reducing cell wall firmness and intercellular adhesion. Softening is attributed to changes in turgor pressure and in the structure and composition of cell walls, such as disassembly of the pectin matrix, mediated at least in part, by the sequential action of pectin methyl esterase and polygalacturonase enzymes (Beaulieu and Gorny, 2004; Pinheiro and Almeida, 2008).

Nutritional aspects have become of interest in the last years. While it has been assume that fresh-cut products would have lowered nutrient content than those marked fresh, some experimental data show different trends depending of type of fruit (Garcia and Barrtett, 2005). Phenolic accumulation is one of the most studied phenomena in response to wounding. Wounding has two effects on phenolic metabolism. The first is the oxidation of endogenous phenolics, released upon cell membrane breakdown, with the enzymes such as PPO, ascorbate oxidase, cytochrome oxidase and peroxidase (Tomás-Barberán and Espín, 2001). The second is the stimulation of cells adjacent to the injury to generate more phenolics in an attempt to initiate repair processes (Salveit, 1997). Reyes et al. (2007) demonstrated that the increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. They measured changes in antioxidant capacity, total soluble phenolics, ascorbic acid content, total carotenoids and total anthocyanins after

wounding in zucchini, white and red cabbage, iceberg lettuce, celery, carrot, parsnips, red radish, sweet potato and potato. The phenolic changes ranged from 26% decrease to an increase up to 191%, while antioxidant capacity changes ranged from a 51% decrease to an increase up to 442%. Reduced ascorbic acid decreased up to 82%, whereas the changes in anthocyanins and carotenoids were less evident (Reyes et al., 2007). Similarly, changes in ascorbic acid content in fresh-cut products are a result of both biosynthesis and degradation reactions during storage (Hu and Jiang, 2007). Increased ascorbate content with time was observed for fresh-cut slices of mangoes kept at 5 and 13 °C, even though the levels never reached that of whole fruit (Tovar et al., 2001). Gil et al. (2006) reported an increase in ascorbic acid content in fresh-cut slices and whole strawberries stored at 5 °C, while a loss of ascorbic acid was observed in pineapple pieces, kiwi fruit slices and in cantaloupe cubes. More recent works show an increase of ascorbic acid in watermelon (Arriola et al., 2014) or in mango cubes (Robles-Sánchez et al., 2013).

Physical stress also results in enzymes coming in contact with substrates, which might contribute to changes in flavour. Such changes are mainly due to the loss of the principal flavour-related volatiles and the synthesis of stress related off-flavour volatiles such as ethanol (Lamikanra et al., 2002; Hodges and Toivonen, 2008). In general, biochemical parameters associated to sugars and acids, such as pH, titratable acidity, soluble solids content and organic and amino acids are important indicators of the overall flavour of fruit and vegetables. However, for fresh-cuts, these parameters are not recommended to be used as quality indicators since they are not significantly affected by storage (Lamikanra and Richard, 2002). For example, the pH, titratable acidity, soluble solid content, organic acids, sugars and amino acids measured in cut cantaloupe after 2 weeks storage at 4 °C were not significantly different from the amounts present in the freshly cut fruit. However, during storage of fresh-cut cantaloupe, the breakdown of esters induced the synthesis of secondary aroma volatile compounds that led to loss of freshness (Lamikanra et al., 2002).

The high water activity of fresh-cut fruits, the level of nutrients and the absence of preservative processes, such as bleaching, freezing or

sterilization, also provide a favourable environment for microbial growth in these products (Dea et al., 2012). Mesophilic and psycrophilic bacteria, yeast and moulds dominate the microflora on fresh-cut fruits and vegetables. During distribution and storage, temperature fluctuations and the high humidity present in packages provide a favourable environment for proliferation of spoilage microorganisms of public health significance (Fan and Song, 2008). In addition, the occasional presence of pathogenic bacteria, parasites, and viruses capable of causing human infections, and therefore, outbreaks of foodborne microorganisms have also been reported (Beuchat, 2002). The pathogens most frequently associated with produce-related outbreaks include bacteria (*Salmonella*, *Escherichia coli* O157:H7), viruses (Norwalk-like, hepatitis A), and parasites (*Cryotosporidium, Cyclospora*), with *Salmonella* and *E. coli* O157:H7 being the leading causes of produce-related outbreaks in the USA (Abadias et al., 2008). *Listeria monocytogenes* is also considered a potential vehicle of foodborne outbreaks caused by the consumption of contaminated minimally processed fresh vegetables (Ryser and Marth, 1991). Moreover, while the acidic pH (3.0 – 5.0) of most fruits restricts microorganism grow, they are not totally without risk, which makes necessary to provide appropriate tools to maintain the product safety within legal limits. The limits established by the Spanish legislation for processed food, including fresh-cut fruits and vegetables, are published in Real Decreto 3484/2000 and they correspond to: 10^6 – 10^7 cfu/g for mesophilic aerobia bacteria; 10 – 10^2 cfu/g for *E. coli* and for *L. monocytogenes* and absence in 25g for *S. enteriditis*.

2.3. Factors affecting quality of fresh-cut fruits

The quality of fresh-cut fruits depends on many factors that include preharvest conditions and postharvest handling. Preharvest factors include the cultivar, cultural practices and the maturity stage at harvest; whereas, postharvest factors include postharvest handling, post-processing treatments, storage conditions (i.e., temperature, humidity, atmosphere), and storage duration (Dea et al., 2012).

2.3.1. Preharvest factors

The selection of the cultivar is of prime importance to assure the optimal quality of a fresh-cut product. Cultivars often differ in sensory, compositional and nutritional qualities and consequently behave differently when processed into fresh-cut products. Depending on the cultivar, different traits may be relevant in selecting cultivars for fresh-cut preparation. Thus for example, while susceptibility to browning is important in fresh-cut apples and pears, there may be more interest in other quality attributes such as texture in kiwifruit, absence of seeds in watermelon or a balance between sweatiness and acidity in pineapple (Garcia and Barrett, 2005). As example of the importance of fruit cultivar, Gorny et al. (1999) reported that the shelf-life of 14 cultivars of peaches and 8 cultivars of nectarines varied between 2 and 12 days at 0 ºC, and their positive response to controlled atmospheres (CA) and to an antibrowning treatment varied greatly.

Many agronomic (soil type, fertilization, water irrigation, etc) and environmental (climate and rainfall) factors also affect fruit quality. However, little has been published in respect to preharvest crop management on the postharvest physiology of fresh-cut fruits. Some recent works show that regulated deficit irrigation strategies (RDI) enabled important savings of water without negatively affecting the quality of fresh-cut nectarines (Falagán et al., 2015) and pomegranates (Peña-Estévez et al., 2015). Furthermore, RDI nectarines had a more stable antioxidant capacity and soluble phenolic content, and showed 10% more vitamin C than the other irrigation treatments (Falagán et al., 2015).

In most fruits, the maturity stage, as determined by harvest date and/or postharvest ripening, is the most important factor that determines storage shelf-life and fruit quality of the processed product. Fruit physiological and metabolic activities change with the ripeness stage. Less mature fruits are expected to maintain better firmness and appearance when processed than ripe fruit, but they are of inferior flavour quality. A mature fruit, on the contrary, will have a superior eating quality but will be more likely to become soft and mealy (Kader, 1999; Gil et al., 2008). For this reason, many works have studied the optimum maturity stage for minimally processed fruits, such as melon

(Oms-Oliu et al., 2007), pear (Bai et al., 2009; Soliva-Fortuny et al., 2004), apple (Soliva-Fortuny et al., 2004; Rojas-Graü et al., 2007), nectarine (Giné-Bordonaba et al., 2014) and guava (Pinto et al., 2010).

2.3.2. Processing handling

The sharpness of the cutting blades used for processing greatly affects the quality attributes of fresh-cut products. A blunt blade will cause accumulation of liquid in the intercellular spaces; which can in turn reduce gas diffusion and induce anaerobic respiration; whereas a sharp blade minimizes tissue damage and associated wound stress responses such as increased respiration and ethylene production (Hodges and Toivonen, 2008). In addition, the cutting shape may also influence the metabolism of fresh-cut tissue, being the surface area of the cut tissue the mean reason (Dea et al., 2012). For example, when stored at 5 °C or 10 °C, slices from fresh-cut papaya had better soluble solid content retention, lower weight loss, and better overall quality index than cubes from the same papaya fruit (Rivera-Lopez et al., 2005) and trapezoidal cuts were shown to extend melon shelf-life compared to slices or cylinders (Aguayo et al., 2004).

2.3.3. Post-processing treatments to extend shelf-life of fresh-cut fruits

Proper temperature management during postharvest handling, processing and distribution is the most important external factor that must be controlled to preserve the quality and safety of fresh-cut fruits and vegetables (Dea et al., 2012). Temperature has a direct relationship with the shelf-life of fresh-cut products. That is, the lower the temperature, the longer the shelf-life of the fresh-cut fruit or vegetable. Generally, recommended storage temperature for these commodities ranges between 3 and 5 ºC, although in some cases retail conditions reach 7 ºC.

As mentioned above, enzymatic browning is one of the most important factors limiting shelf-life of fresh-cut products. The classic methodology to inhibit enzymatic browning is the use of antioxidants. Table 2 shows the types of chemicals that are used to control browning. Some types act directly as inhibitors of PPO, others render the medium inadequate for the development of the browning reaction, and still others

react with the products of the PPO reaction before these can lead to the formation of dark pigments (Garcia and Barrett, 2002).

Table 2. Chemical agents with inhibitory action on enzymatic browning

Browning inhibitor	**Effect/ Action**
Acidulants	
Citric acid	Possible dual effect: lowering pH and chelating Cu^{+2} from PPO active site
Other organic acids: Tartaric acid, malic acid, lactic acid	Lower pH
Inorganic acids: phosphoric acid, phydrochloric acid	Lower pH
Reducing agents	
Ascorbic acid	Reduction of *O*-quinones to colorless diphenols
Erythorbic acid	Same as ascorbic acid
Ascorbyl-phosphate esters	Release ascorbic acids upon hydrolysis by acid phosphatase present in plant tissue
Sulfhydryl comounds: L-cysteine	React with *O*-quinones producing colorless stable products
Complexing agents	
Cyclodextrins (Cyclic oligosaccharides): β-cyclodextrin	Formation of complexes with PPO substrates Entrapment of PPO substrates or products
Chelating agents	
EDTA	Metal chelator: Binds Cu^{+2} at the PPO active site and Cu^{+2} available in the tissue
Polyphosphate	Chelator Chelator and acidulant
Sodium acid: Sodium hexametaphosphate	
Enzyme inhibitor	
4-hexyl resorcinol	PPO inhibitor
Chloride anions (NaCl, $CaCl_2$, $ZnCl_2$)	Interaction with Cu^{+2} at the PPO active site

Source: García and Barrett (2002)

Among them, ascorbic acid, its isomer (*d*-isoascorbic acid) and citric acid are probably the most common antibrowning agents selected for use in fresh-cut products. Thiol-containing amino acids such as N-acetylcysteine (Oms-Oliu et al., 2006; Rojas-Graü et al., 2006a), chelators such as EDTA and cyclodextrine (Pilizota and Sarpers, 2004), or compounds that directly inhibit PPO such as 4-hexyl resorcinol (Arias et al., 2007) have also been investigated to prevent browning of several fresh-cut fruits and vegetables. The latest, although they are not specifically approved by legislation for use in fresh fruits and vegetables, they are approved as food ingredients for other foods (i.e. Generally Regarded as Safe compounds (GRAS) and food-grade additives by the U.S.A and European legislations, respectively).

The numerous works that can be found in the literature show that the effectiveness of antioxidants to control browning depends on many factors such as fruit product, cultivar, maturity stage, concentration, synergy with other antioxiants and/or other physical treatments, pH, application systems, etc (Garcia and Barrett, 2005). Thus for example, ascorbic acid, although being effective in many fresh-cut fruits, confers a temporary protection as it is oxidized in the process of preventing browning. Moreover, the combination of different antioxidants has also shown to have a synergetic effect to reduce browning on fruits. For example, the application of ascorbic acid and 4-hexyl resorcinol had a synergic effect preventing enzymatic browning on fresh-cut 'Conference' pears (Arias et al., 2007).

Softening is a quality defect that also compromises the shelf life of many fresh-cut fruits that are perceived by consumers as crunchy or firm. Although selection of appropriate fruit cultivars and maturity stage at harvest is essential, the use of calcium salts is a common practice to reduce softening. The firming effect of calcium is attributed to the formation of cross-links with cell wall and middle lamella pectins (Luna-Guzmán et al., 1999; Rico et al., 2007). On the other hand, calcium chloride ($CaCl_2$) treatments have also been reported to reduce browning, which can be attributed to the PPO inhibition by the chloride anion (Varela et al., 2007). Various studies have also shown the effectiveness of ascorbic acid or citric acid mixed with $CaCl_2$ to extend the shelf life of

fresh-cut apples (Rocha et al., 1998; Tortoe et al., 2007; Chiabrando and Giacalone, 2012) or pears (Soliva-Fortuny et al., 2004).

The use of 1-MCP has also been proven effective to slow the changes associated with loss of firmness and to extend the shelf-life of fresh-cut fruits such as kiwifruit, mango and persimmon (Vilas-Boas and Kader, 2007), melon (Ergun et al., 2007), apples (Perera et al., 2003; Calderón-López et al., 2005) and pears (Arias et al., 2009; Lu et al., 2009). Response of fresh-cut products to 1-MCP treatment depends on the dose applied, the type of crop, the maturity or the ripeness stage, the exposure time, and the temperature (Blankenship and Dole, 2003).

Successful applications of modified atmosphere packaging (MAP) with low O_2 and high CO_2 for fresh-cut fruits and vegetables have been extensively reported in the literature (Rojas-Graü et al., 2009; Caleb et al, 2013). The beneficial effect of MAP to maintain quality of minimally processed fruits is related to a reduction in respiration rate and ethylene production, water loss, phenolic oxidation, and aerobic microbial count. However, the beneficial effects of MAP on the quality of fresh-cut fruits and vegetables depend upon a number of uncontrollable factors, such as the species, cultivar, cultural practices, maturity stage, postharvest handling, as well as controllable factors, including packaging material gas permeability, respiration rate, and storage conditions (Kader and Ben-Yehosua, 2000). Furthermore, exposure to O_2 or CO_2 levels outside the limits of tolerance may lead to anaerobic respiration with the production of undesirable metabolites and other physiological disorders (Oms-Oliu et al., 2007).

MAP requirements and recommendations for fresh-cut fruits report low O_2 (1–5 kPa) and/or elevated CO_2 (5–10 kPa) levels to maintain quality and consequently extend shelf life of many products (Gorny, 2003). In some cases, displacing the air within the package with a known mixture of gases close to the recommended atmospheres (active MAP) helps to extend the shelf life of the cut product compared to the use of passive MAP by reducing the metabolism, delaying browning reactions, and inhibiting microbial growth as soon as the product is cut. Thus for example, fresh-cut honeydew and cantaloupe packaged in active MAP with 5 kPa O_2 + 5 kPa CO_2 and 4 kPa O_2 + 10 kPa CO_2, respectively, had better colour retention, reduced respiration rate and microbial population,

and longer shelf-life than those in passive MAP (Bai et al., 2001, 2003). In fresh-cut pears and melon, packaging under 2.5 kPa O_2 + 7 kPa CO_2 inhibited ethylene synthesis (Soliva-Fortuny et al., 2007; Oms-Oliu et al., 2008a, 2008b, 2009). However, O_2 consumption and CO_2 production rates of just-packaged fresh-cut pears were stimulated more significantly under 2.5 kPa O_2 and 7 kPa CO_2 atmospheres than under initial 21 kPa O_2 (Oms-Oliu et al., 2008b). This phenomenon was attributed to a possible effect of the vacuum created in packages before flushing the gas mixture, promoting changes in the pear tissue structure, as well as a dramatic modification of the internal atmosphere. On the contrary, fresh-cut pears stored in 21 kPa O_2 did not suffer such stress due to vacuum. Therefore, it is important to experimentally determine the appropriate gas composition for each particular product and handling/processing conditions (e.g. processed form, package format, storage conditions, etc.).

In products rich in antioxidant compounds the only use of low O_2 and high CO_2 MAP may be insufficient to prevent browning and provide sufficient shelf life to the cut product. Therefore, most of the works in the literature report the combine effect of antioxidants and MAP to control enzymatic browning and extend the shelf life of various fresh-cut fruits as mango (González-Aguilar et al., 2000), pear (Gorny et al., 2002), apple (Rocculi et al., 2004), strawberry (Aguayo et al., 2006), banana (Vilas-Boas and Kader, 2006), among others. In fresh-cut papaya, the combination of passive MAP (5 kPa O_2 + 10 kPa CO_2) and the antioxidant dip ($CaCl_2$ and citric acid) improved colour, maintained firmness, reduced microbial count, and extended the shelf life of the product up to 25 days at 5 ºC, whereas the treatments alone were not effective in preserving fruit quality (Waghmare and Annapure, 2013). Furthermore, the combination of antioxidants and passive MAP showed a significantly lower decrease in O_2 concentration in the package and correspondingly lower increase in CO_2, avoiding off-flavour development.

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 proving a semipermeable barrier to water vapour and gas exchange.

Therefore, edible coatings can contribute to extend shelf life of fresh-cut fruits by reducing moisture loss, respiration rate as they create a modified atmosphere and enzymatic browning. According to the European Directive (ED, 1995, 1998) and USA regulations (FDA, 2006), edible films and coatings can be classified as food products, food ingredients, food additives, food contact substances, or food packaging materials. The development of edible films and coatings has been focused upon barriers containing proteins as whey, casein, soy protein; polysaccharides as chitosan, alginate, cellulose, carrageenan, pectin, starch; and lipids as carnauba, beeswax and fatty acids. The functionality of the coatings depends on the properties of their compounds, the final composition and the fruit product. Several works have described the beneficial effect of polysaccharide and protein-based edible coatings on reducing the respiration rate of fresh-cut produces, which has been attributed to their good oxygen barrier. Hence, lower CO_2 production has been observed in fresh-cut apples, melons, and pears coated with an alginate-based edible (Rojas-Graü et al., 2007; Oms-Oliu et al., 2008c; Raybaudi-Massilia et al., 2008a, 2008b) and in apple slices coated with whey protein (Lee et al., 2003).

The functional properties of edible coatings for fresh-cut fruits are usually enhanced by the incorporation of active ingredients such as antioxidants, texture enhancer, and antimicrobials to reduce enzymatic browning, texture loss, and the risk of pathogen growth on food surfaces. Thus, fresh-cut pears were preserved from surface browning by a methyl cellulose coating containing ascorbic acid (Olivas et al., 2003) or alginate and gellan coatings containing N-acetylcysteine and glutathione (Oms-Oliu et al., 2008c). Browning of fresh-cut apples has also been controlled or reduced by carrageenan and whey protein-based coatings containing ascorbic acid, citric acid, cysteine or oxalic acid (Lee et al., 2003; Pérez-Gago et al., 2006), alginate and gellan coatings containing N-acetylcysteine (Raybaudi-Massilia et al., 2008a; Rojas-Graü et al., 2008) and chitosan coatings containing ascorbic acid (Qi et al., 2011). Furthermore, the adding of calcium as a gelling agent to alginate, gellan and pectin-based edible coatings have also shown to be effective at maintaining firmness in several fresh-cut products such as apple (Rojas-Graü et al., 2007, 2008; Freitas et al., 2013; Pan et al., 2013), pineapple (Azarakhsh et al. 2014) and melon (Raybaudi-Massilia et al., 2008b).

Microbiological stability is also a critical factor to maintain the commercial marketability of fresh-cut fruits. Washing with chlorinated water is the most widely employed sanitation procedure. Even though the application of chlorine is not considered very effective in reducing microbial levels in contaminated tissue, chlorine reduces microbial loads in the water and prevents cross-contamination (Dea et al., 2012). However, there is some controversy about using chlorine as an antimicrobial agent due to the possible formation of carcinogenic chlorinated compounds in the rinsing water, namely chloramines and trihalomethanes (Rico et al., 2007). Therefore, many studies focus on finding new alternatives to chlorination, which includes the use edible coatings with antimicrobial activity (Valencia-Chamorro et al., 2011). Among the different coatings, chitosan has been widely tested in fresh-cut fruits and vegetables for its antimicrobial property (González-Aguilar et al., 2009; Moreira et al., 2011; Qi et al., 2011). Incorporating antimicrobial compounds into edible coatings has been another approach to enhance the safety and extend the shelf life of fresh-cut products. Several antimicrobial compounds have been investigated as for incorporation into edible coatings, including organic acids (acetic, benzoic, lactic, propionic, sorbic), fatty acid esters (glyceryl monolaurate), polypeptides (lysozyme, peroxidase, lactoferrin, nisin), and plant essential oils (cinnamon, oregano, lemongrass). The use of these coatings 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 (Valencia-Chamorro et al., 2011). The effectiveness of different antimicrobial substances such as lysozyme, nisin, organics acids, essential oils and their derivatives incorporated into the edible films have showed to be satisfactory against several pathogens (Eswaranandam et al., 2004; Cagri et al., 2004; Pranoto et al., 2005; Oussualah et al., 2006; Rojas-Graü et al., 2006b). 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).

Some attempts have also been focused to extend the shelf life of fresh-cut fruits by combining edible coatings and MAP technologies.

Mastromatteo et al. (2011) studied the effect of a sodium alginate edible coating enriched with a hydro-alcoholic solution and grape seed extract and two MAP conditions (passive and active MAP with 10 kPa O_2 + 10 kPa CO_2) on the quality of minimally processed kiwifruits. The combination of active compounds with alginate-based coating was more effective at delaying the microbial growth than the active compounds alone and reduced weight loss and respiration rate of the product. Overall, the alginate-based coating increased the shelf life of the samples up to 14 and 12 days when packed in active and passive MAP, respectively, compared to 8 days for the control samples. Similarly, a chitosan coating showed a high antimicrobial activity at inhibiting the growth of psychrotrophic and mesophilic bacteria, yeast and moulds of fresh-cut strawberries at temperatures as high as 15 °C. The use of MAPs with high (80 kPa) and low (5 kPa) O_2 concentrations as second hurdle technology resulted in a sensible benefit on the microbiological quality of fresh-cut strawberries. Conventional MAP contributed to better control psychrotrophic bacteria; whereas high oxygen concentrations had a beneficial effect on sensorial characteristics, colour and at inhibiting mesophilic bacteria.

3. Research on fresh-cut persimmon

In the literature, few works have focused in the study of fresh-cut persimmon. The first works studied the effect of controlled atmosphere storage in non-astringent 'Fuyu' persimmon held for 8 days at 5 ºC. Persimmon slices maintained good visual quality up to day 8 of storage under the various atmospheres (Wright and Kader, 1997a). Fruit stored under air + 12 kPa CO_2 or 2 kPa O_2 + 12 kPa CO_2 controlled atmospheres were still marketable at the end of the study; whereas areas of faint black pigmentation had begun to develop on the fruit stored in air or 2 kPa O_2 and were therefore judged to be at the limit of marketability by day 8 of storage. This cultivar also showed a loss of total vitamin C in the first day after cutting, but then recovered to levels not significantly different from the initial and at the end of the 8 days no differences among treatments were found (Wright and Kader, 1997a). On the other hand, there were no clear trends in the content of major carotenoids during storage in the different controlled atmospheres, although persimmon slices tended to lose total retinol equivalent content over 8

days of storage (Wright and Kader, 1997b). In later studies with the same cultivar, the treatment with 1-MCP (1_L L^{-1} at 10 ºC for 6 h) prevented the softening of fresh-cut 'Fuyu' persimmons stored for 7 days at 5 ºC when applied on intact fruit before processing, but not when applied in fresh-cut persimmons (Vilas-Boas and Kader, 2007). Colour L* values declined rapidly after processing and no differences were found between 1-MCP treated and untreated samples. The beneficial effect of 1-MCP treatment on preserving fruit firmness of fresh-cut persimmon was also reported for the astringent cultivar 'Saijo' (Itamura et al., 2009).

Yukari et al. (2012a) evaluated the physicochemical quality of non-astringent 'Fuyu' and astringent 'Tonewase' persimmon cultivars enzymatically peeled for fresh-cut slices. The results indicated that enzymatic peeling could be an alternative to knife-peeling of 'Tonewase' persimmon fruit for fresh-cut production, as the colour index, pH, and texture were unaffected by enzymatic peeling, but not for 'Fuyu' persimmon. Furthermore, when microbial contamination of enzyme-peeled and knife-peeled persimmon slices was compared, the bacterial counts and diversity of bacterial and fungal flora were less in enzyme-peeled slices than in knife-peeled slices. Later studies recommended a 20% CO_2 atmosphere storage for reducing the microbial population of enzyme-peeled persimmon slices stored at 10 ºC and the shelf life of 'Tone-wase' persimmon slices in an active MAP with 20% CO_2 was 4 days (Yukari et al., 2012b). Similar studies in freshly peeled 'baby persimmon' cv. 'Totsutanenashi' confirmed these results. Although microflora was decreased in 20% CO_2 atmosphere as compared to air on day 6 all samples developed browning to an unmarketable level and the maximum shelf life was stabilised by day 4 of storage at 10 ºC (Izumi et al., 2015).

In fresh-cut 'Rojo Brillante' persimmon, Ghidelli et al. (2013) evaluated in *in vitro* studies (extracts and precipitates) the potential to control enzymatic browning of a wide range of antioxidant agents at different concentrations. Then, the most effective antioxidants were studied on fresh-cut tissue during storage at 5 ºC. Ascorbic acid and citric acid were the most effective treatments in reducing enzymatic browning of fresh-cut persimmon, reaching the limit of marketability in the range of 5-7 days. In particular, concentrations of 1.12% ascorbic acid and

0.21% citric acid seemed to be the most effective in controlling enzymatic browning of fresh-cut persimmon slices. $CaCl_2$ also contributed to extending the shelf-life of persimmon pieces; however its effectiveness was much lower than ascorbic acid and citric acid. The combination of an optimized soy protein isolate-based edible coating with ascorbic acid as antioxidant and MAP was also evaluated in 'Rojo Brillante' persimmon (Ghidelli et al., 2010). MAP conditions included active conventional MAP (5 kPa O_2 + 15 kPa CO_2), passive MAP, and high O_2 MAP (>50 kPa, balanced N_2) and they were compared to atmospheric conditions as control. Among the different conditions studied, the combination of the coating with the conventional active MAP was the most effective treatment to control tissue browning of fresh-cut 'Rojo Brillante' persimmon and persimmon slices reached a limit of marketability of 8 days at 5 ºC. More recently, Almela et al. (2014; 2015) reported that the application of calcium lactate, MAP (5% and 10% CO_2) or essential oils had a moderate effect on respiration rate, colour and firmness of 'Rojo Brillante' persimmon slices, but no information was provided about shelf life of treated samples versus untreated ones.

Considering that astringent cultivars require appropriate technologies to remove astringency before being minimally processed, Chung et al. (2015) studied the effect of different pre-slicing deastringency treatments (non-treated, CO_2 gas, warm water, ethanol vapour) on the quality of fresh-cut 'Cheongdobansi' persimmons during 6 days of storage at 10 ºC. From these treatments, ethanol vapour negatively affected flesh firmness of the persimmons; whereas, warm water treatment was found to be the most effective in inhibiting surface browning and in controlling flesh softening in fresh-cut persimmons.

The above sections show the importance for an integrated approach to obtain fresh-cut fruits. Exploration of technologies as MAP and edible coatings in highly perishable fruits that have received little or no attention such as 'Rojo Brillante' persimmon is required to increase the offer of fresh-cut fruits, providing producers and consumer's products with sufficient shelf life and the maximal safety and quality. This also requires an integrated approach that would consider the quality of the raw product. All the factors should be seen as a way of ensuring not only

extended shelf life, but also a convenient high quality product that it is safe, nutritionally sound, and appealing to the senses.

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GENERAL OBJECTIVES

GENERAL OBJECTIVES

To develop optimum procedures for processing and marketing 'Rojo Brillante' persimmon into a fresh-cut product with the maximum shelf life and best physicochemical, nutritional, sensory and microbiological quality.

Specific objectives

1. To determine the effect of ripeness stage at harvest, storage time at 15 ºC before processing and antioxidant dips on the physicochemical, sensory and nutritional quality of fresh-cut persimmon. To establish maximum shelf life of the fresh-cut product as affected by these factors.

2. To use controlled atmosphere (CA) storage of fresh-cut persimmon slices to determine the optimal reduced O_2 and/or elevated CO_2 concentrations that optimize the shelf life of antioxidant treated persimmon slices. To study their effect on tissue browning and softening, visual quality, and bioactive compounds.

3. To evaluate the effect of antioxidant dips and modified atmosphere packaging (MAP), using the optimal atmosphere that was determined in the previous objective, in the control of enzymatic browning of fresh-cut 'Rojo Brillante' persimmon.

4. To study the effects of postharvest application of 1-Methylcyclopropene (1-MCP), storage time at 1 ºC before processing, and postcutting antioxidant treatment on shelf life of fresh-cut 'Rojo Brillante' persimmons.

5. To develop new edible coatings amended with antioxidant and antimicrobial agents to reduce enzymatic browning and control microbial growth of fresh-cut 'Rojo Brillante' persimmon. To study the effect of antimicrobial coatings on

the survival of important food-borne human pathogens artificially inoculated on fresh-cut fruit during storage at 5 ºC.

6. To evaluate the combined effect of the optimized edible coating and MAP on the physicochemical and sensory quality and microbial growth of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC.

Chapter 1

Effect of maturity stage at processing and antioxidant treatments on the physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmon

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ABSTRACT

The aim of this work was to study the effect of the maturity stage (MS), storage at 15 ºC before processing and antioxidant application on the quality of fresh-cut 'Rojo Brillante' persimmons. The MS at harvest had an effect on both fruit firmness and the efficacy of the antioxidants to control enzymatic browning. Nutritional quality was affected by MS and storage time before processing, but not by antioxidant application. For commercial purposes, the persimmons harvested at the beginning of the season could be processed as a fresh-cut commodity, even after 3 days of storage at 15 ºC if treated with 0.01 kg L^{-1} ascorbic acid (AA) or 0.01 kg L^{-1} citric acid (CA). However, processing fruits from late season immediately after harvest and being treated with AA are recommended. The limit of marketability of fresh-cut 'Rojo Brillante' persimmons was reached after 6 and 8 days with AA and CA, respectively.

KEYWORDS: Minimally processed, persimmon, maturity stage, phenolic content, radical scavenging activity, carotenoids.

1. Introduction

Persimmon (*Diospyros kaki* L.) 'Rojo Brillante' is an astringent variety characterised by good growing conditions, excellent colour, size, sensory characteristics and good nutritional properties. In the last decade, its production has grown substantially in Spain given the application of high levels of CO_2 to remove astringency while firmness is preserved (Salvador et al., 2007). This technology has also increased its potential as a fresh-cut commodity. However processing operations, such as peeling and cutting, cause damage to tissue and increased physiological activity, which lead to major physicochemical changes, such as browning, off-flavour development and softening that significantly reduce the fruit´s shelf life.

The response of each fruit to processing may be affected by many factors, such as cultivar, maturity stage at processing, post-cutting treatments, and storage conditions (Gil et al., 2006). In most fruits, the maturity stage (MS), as determined by harvest date and/or postharvest ripening, appears essential for maintaining the quality and for accomplishing an appropriate shelf life of fresh-cut produce (Soliva-

Fortuny et al., 2002). Furthermore, the effects of the MS and wounding processing on bioactive compounds of fruits are directly linked. Thus carotenoids and ascorbates are unstable when exposed to acidic pH, oxygen or light, all of which may occur when cells are disrupted by cutting (Wright and Kader, 1997). Among the different technologies used to extend the shelf life of fresh-cut fruits, antioxidants as the main approach are employed to inhibit enzymatic browning. In recent works on fresh-cut 'Rojo Brillante' persimmon, ascorbic acid (AA), citric acid (CA) and cysteine (Cys) proved to be the most effective antioxidants to control enzymatic browning, and they helped a limit of marketability of 5-7 days of storage at 5 ºC to be achieved (Ghidelli et al., 2013). The same studies also showed some antioxidant activity for $CaCl_2$, which together with its potential to preserve fresh-cut persimmon from texture loss, makes it an interesting alternative to extend shelf life.

Although several works have described the optimum maturity stage for minimally processed fruits, such as melons, pears and apples (Soliva-Fortuny et al., 2002, 2004; Oms-Oliu et al., 2007; Rojas-Graü et al., 2007), no works can be found for persimmon fruits. Persimmon cv. 'Rojo Brillante' has a harvest period of about 2 months, with production taking place between October and November. Storage at 15 ºC can extend the limit of storability in packing houses to periods lasting no longer than 20 days due to progressive softening. Several works have described changes in phenolic compounds, such as flavonoids, tannins and phenolic acids (Del Bubba et al., 2009; Oz and Kefalas, 2010), total antioxidant capacity (Chen et al., 2008; Del Bubba et al., 2009), carotenoid content (Giordani et al., 2011) and fruit firmness (Salvador et al., 2007), during the on-tree maturation and postharvest storage of persimmon fruits. However, very few works have studied the effect of cutting on the commercial shelf life and bioactive compounds of fresh-cut persimmon. Wright and Kader (1997) showed a loss in vitamin C and carotenoid content in fresh-cut 'Fuyu' persimmons stored in controlled atmospheres after cutting. A preliminary study by our group observed that the MS affected the shelf life of fresh-cut 'Rojo Brillante' persimmons (Sanchís et al., 2012). Therefore, the objective of this work was to evaluate the effect of the MS at harvest, the storage time at 15 ºC before processing and the application of antioxidant treatments on the physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmons.

2. Materials and methods

2.1. Reagents and solvents

Ascorbic acid (AA) and citric acid (CA) were supplied from Quimivita (Barcelona, Spain). Cysteine (Cys), calcium chloride ($CaCl_2$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, sodium chloride, ammonium acetate, potassium hydroxide, β-apo-8'-carotenal and β-carotene were obtained from Sigma (St. Louis, MO, USA). Methanol, chlorhydric acid, ethanol, hexane, methylene chloride, acetonitrile and butylated hidroxytoluene were purchased from Scharlau (Barcelona, Spain). β-cryptoxanthin, lutein, lycopene and zeaxanthin were provided from Extrasynthese (Genay, France). Gallic acid was acquired from Acros Organics (Geel-Belgium) and thriethylamine from Panreac (Barcelona, Spain). All the solvents used were HPLC-grade and Milli-Q system ultrapure water (Millipore Corp., USA) was used throughout.

2.2. Plant material

Persimmon fruits (*Diospyros kaki* cv. Rojo Brillante) were provided by the Protected Designation of Origin (PDO) Kaki Ribera del Xúquer (Valencia, Spain) with two different commercial maturity stages (MSs) determined by external colour as MS1 with a colour index (CI) of 1.5 and MS2 with a CI of 17.6, where CI=1,000 *a/L b*. These MSs were from fruits harvested in early October and mid-November, which corresponded to the beginning and end of the season respectively. The fruits from both MSs were stored for 0, 3 and 6 days at 15 ºC before processing (S-0, S-3 and S-6). Astringency was removed by applying 95% of CO_2 in closed containers for 24 h at 20 ºC and 90% RH. The physicochemical characteristics of persimmon fruits before processing were evaluated in 30 fruits for soluble solids content (Atago Pocket refractometer, Atago company Ltd., Japan), total acidity (Titration Excellence T50, Mettler Toledo, Barcelona, Spain), external colour (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Osaka, Japan) and firmness (Instron Universal Machine, Model 3343, Instron, Barcelona, Spain). External colour, expressed as CI, was calculated using the Hunter *L*, *a*, *b* colour space, and fruit firmness was the maximum

force in newtons (N) required to penetrate fruit flesh after removing the skin on the equator.

2.3. Sample preparation

Persimmons were washed with chlorinated water (150 mg L^{-1}), peeled and cut into rectangular pieces (approx. 5 cm x 3.5 cm x 1.5 cm) with a sharp stainless steel knife. Pieces were dipped into antioxidant solutions for 3 min and were allowed to drain and dry at 5 ± 1 ºC. The tested antioxidant solutions were: 0.01 kg L^{-1} CA, 0.01 kg L^{-1} AA, 0.005 kg L^{-1} Cys and 0.005 kg L^{-1} $CaCl_2$. Control pieces were dipped in distilled water under similar conditions. Once dried, four pieces (115 ± 10 g) were placed on polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and were heat-sealed with microperforated polypropylene films (35-µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure that the atmosphere on the tray was not modified, and to study the effect of only the antibrowning agents, the film was 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). Finally, samples were stored for 8 days at 5 ± 1 ºC. No more than 15 persimmons were processed at the same time to minimize their exposure to oxygen. The whole process was carried out in a temperature-controlled room at 5 ± 1 ºC under suitable hygienic conditions. Nine trays were prepared per treatment and sampling period to determine colour, sensory and nutritional quality. For nutritional quality, samples were frozen in liquid nitrogen and stored at –80 ºC until analysed.

2.4. Colour evaluation

The colour of persimmon pieces was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan), calibrated with a standard white plate, on 12 pieces per treatment using the CIE *L* a* b** colour parameters. Each measurement was taken randomly at three locations per sample piece.

2.5. Firmness measurements

The firmness of fresh-cut persimmons 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 and at a speed of 5 mm/s. Twelve samples per treatment were measured and the results are expressed in newtons (N).

2.6. Sensory quality

The sensory quality of persimmon slices was conducted by 15 trained judges, and included a visual and taste evaluation. For the visual test, each treatment was presented on trays that contained 12 persimmon pieces to account for sample variability, labelled with a 3-digit random code and presented to the judges under the same conditions (light intensity and temperature) to minimise variations in human perception. Visual quality, based on the 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 the samples.

The taste evaluation included the overall flavour, off-flavours, and firmness of the fresh-cut persimmons. Overall flavour was rated on a 9-point scale, where 1-3 represented a poor quality range, 4-6 an acceptable quality range, and 7-9 an excellent quality range. Off-flavour was rated on a 5-point scale, where 0 = absence and 5 = marked presence. Firmness was rated on a 5-point scale, where 1 = very soft, 3 = neither firm nor soft, and 5 = very firm. These scales permitted us to discriminate each attribute, and they were easily understood and unbiased (ISO 4121:2003). Two persimmon slices, selected randomly from each treatment to compensate for the biological variation of material, were presented to the panelists on trays labelled with 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.

2.7. Free radical scavenging activity analysis

The antiradical capacity of the persimmon slices was determined by the method of Brand-Williams et al. (1995), using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) as the free radical. Extraction was done as described by Chen et al. (2008) with some modifications. Two grams of persimmon pulp were mixed with 30 mL of 80% methanol. The solution was homogenised at 20,000 rpm for 2 min (Ultraturrax, IKA, Germany), followed by boiling in a water bath for 20 min to inactivate the PPO enzyme. The homogenate was immersed in an ultrasonic machine at room temperature for 15 min and centrifuged at 10,000 rpm for 20 min at 5 ºC. The resultant supernatant was then filtered and used as the persimmon extract. A second pulp extraction was needed to complete the extraction. The mixture of both extracts was used to analyse the samples' antiradical capacity.

Five methanolic dilutions from the supernatant were prepared to relate the decrease of $DPPH^{\bullet}$ absorbance with sample concentration. Seventy five μL of extract were mixed with 225 μL of $DPPH^{\bullet}$ (24 ppm), and the mixture was kept in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 520 nm using a multiplate spectrum (Multiskan Spectrum, Thermo Fisher Scientific, Finland). $DPPH^{\bullet}$ radical scavenging activity was expressed as the amount of persimmon extract needed to reduce the initial $DPPH^{\bullet}$ concentration by 50% (EC_{50}); thus lower EC_{50} values mean greater antiradical capacity. Radical scavenging activity was expressed as g of persimmon fruit per kg of $DPPH^{\bullet}$. Three replicates per treatment were determined.

2.8. Total phenolic content (TPC)

TPC was measured following the method described by Chen et al. (2008). One gram of sample was mixed with 15 mL of methanol with 0.01 mL L^{-1} hydrochloric acid. This mixture was homogenised at 10,000 rpm for 1 min, immersed in an ultrasonic bath for 30 min and centrifuged at 12,000 rpm for 20 min at 4 ºC. The supernatant was filtered and collected. The extraction was repeated and supernatants were combined for the analysis. Two methanolic dilutions were prepared with the extracts. Next 300 μL of supernatant were mixed with 600 μL of Folin Ciocalteu reagent and 2.4 mL of sodium carbonate solution (200

mg/mL), and in this order. The mixture was incubated for 1 h in the dark at room temperature. The absorbance of the resulting solution was measured at 765 nm using the spectrum multiplate reader. The results were expressed as mg of gallic acid per 100 g of persimmon fruit. Three replicates per treatment were determined.

2.9. Carotenoids

The extraction, saponification and quantification methods were performed as described by Wright and Kader (1997). For the extraction, 5 g of sample were added to a centrifuge tube together with 10 mL of cold ethanol to be homogenised (Ultraturrax, IKA, Germany) for 3 min at 16,000 rpm. 8 mL of hexane were added and the sample was homogenised for another 2-minute period. The mixture was then centrifuged for 4 min at 7000 x g and 4 ºC. The organic phase was transferred to a 250-mL screw-cap Erlenmeyer flask with a pasteur pipette. 5 mL of saturated sodium chloride were added to the centrifuge tube contents and stirred gently. An additional 8 mL of hexane were added, and the mixture was homogenised for 1 min and centrifuged under the same conditions as those described above. The resultant organic phase was then transferred to the Erlenmeyer flask with the first extract.

For the saponification, 15 mL of 10% methanolic potassium hydroxide were added to the Erlenmeyer flask, which was flushed with nitrogen, sealed and covered with aluminium foil to prevent oxygen and light. Then the flask was left at room temperature with gentle shaking for 16 h. Next the mixture was transferred to a separatory funnel to remove the potassium hydroxide with 15 mL of 10% sodium chloride, followed by deionised water until the mixture had a neutral pH. The potassium hydroxide from the vial was extracted with an additional 10 mL of hexane. Both hexane extracts were evaporated under nitrogen until dryness and were kept at –80 ºC until analysed. At the time of the analysis, samples were redissolved in 200 μL of methylene chloride and 1.8 mL of the mobile phase. Major carotenoids were determined by high-performance liquid chromatography (HPLC) in three replicates per treatment.

The resuspended sample (1.5 mL) was filtered though a 0.45-μm nylon filter into amber vials before being analysed by HPLC. The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200) quaternary pump (Model L-2130), column oven (Model L-2300) and diode array detector (Model L-2450). A reversed-phase C30 YMC-Pack column (250 x 4.6 mm, 5 μm-particle, Merck, Darmstadt, Germany) was used. The injection volume was 60 μL and the oven temperature was 4 ºC. The mobile phase consisted of acetonitrile, methanol containing 0.05 mol L^{-1} ammonium acetate and methylene chloride 75:20:5 v/v/v, containing 1 mL L^{-1} butylated hydroxytoluene and 0.5 mL L^{-1} triethylamine. The flow rate was 1.5 mL/min. Detection was performed at 450 nm. Identification of peaks was confirmed using standards of major compounds. The retinol equivalent (RE) was calculated on the basis of 1 RE = 6 μg of β-carotene or 12 μg of other provitamin A carotenoids (β-cryptoxanthin and α-carotene).

2.10. Statistical analysis

Statistical analyses were performed with STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences among treatments were determined by least significant differences (LSD) after the analysis of variance (ANOVA). One- and two-way ANOVAS were performed for the effect of the main factors (antioxidant treatment (AT), maturity stage (MS) and storage (S) time of persimmons at 15 ºC before processing) and their interactions on the different quality parameters. Significant differences were defined at $p \leq 0.05$.

3. Results and discussion

3.1. Physicochemical characteristics of whole persimmon fruit

The physicochemical characteristics of 'Rojo Brillante' persimmons before processing are shown in Table 1. The averages of the total soluble content and total acidity of persimmons were 16.09 ± 0.51 ºBrix and 0.65 ± 0.05 g malic acid/100 g, respectively. These parameters were not significantly affected by the MS or the storage time at 15 ºC before processing. The fruits harvested at MS2 had a higher CI and less firmness

than those harvested at MS1. Furthermore, fruit firmness decreased and CI increased as the storage time at 15 ºC before processing prolonged from S-0 to S-6 for both the MSs.

3.2. Colour changes

The tissue browning of the persimmon slices processed at the two MSs after 0, 3 and 6 days of storage at 15 ºC was reflected mostly by changes in L* (lightness) and a* (redness) (Figs. 1 and 2). The ANOVA F-ratios for the effect of the main factors and their interactions on these colour parameters are shown in Table 2. The antioxidant treatment and the MS had a significant effect on L* and a*, whereas the storage time at 15 ºC before processing had an effect only on the L* values. Significant interactions were observed between factors, except between antioxidant treatment and the storage time at 15 ºC on the a* values. These results indicate the importance of selecting the right antioxidant according to the MS at harvest for processing 'Rojo Brillante' persimmons.

At harvest, the persimmons with MS1 had higher L* and lower a* values than those with MS2. This can related to an increase in carotenoid content as the MS advances, which is an important factor that influences changes in colour (Zhou et al., 2011). Upon processing, increased enzymatic browning of the fresh-cut persimmons during the storage at 5 ºC was accompanied by an increase in the a* and a decrease in the L* values. At MS1, the control samples had a lower L* and a higher a* than the antioxidant-treated samples, irrespectively of the storage time at 15 ºC of persimmon fruits before processing. In all cases, the main change in the L* and a* values was observed after 1 day of processing, and they remained constant afterwards during the storage at 5 ºC. Among the antioxidant treatments, AA and CA provided the lowest a* values, which indicates greater browning inhibition, whereas Cys was the antioxidant that provided the highest L* values. This result confirmed previous findings by Ghidelli et al. (2013) in fresh-cut Rojo Brillante persimmons, who reported that AA and CA were the most effective treatments from among the several antioxidants that were studied to reduce enzymatic browning.

Table 1. Physicochemical characteristics of persimmon cv. 'Rojo Brillante' before processing.

	MS1			MS2		
	S-0	S-3	S-6	S-0	S-3	S-6
Soluble solids (ºBrix)	16.5 ± 0.2	16.48 ± 0.3	16.3 ± 0.3	15.1 ± 0.6	16.3 ± 0.2	16.0 ± 0.3
Total acidity (g malic acid/100 g)	0.59 ± 0.01	0.69 ± 0.03	1.49 ± 0.03	0.70 ± 0.04	0.58 ± 0.05	0.70 ± 0.01
CI (1000 *a* / *L b*)	1.5 ± 1.0	5.4 ± 2.0	5.8 ± 2.4	17.6 ± 1.8	22.8 ± 2.0	21.2 ± 2.2
Firmness (N)	45.6 ± 4.7	47.0 ± 4.7	37.5 ± 5.0	20.9 ± 5.4	16.8 ± 5.1	13.2 ± 4.6

Values are mean ± standard deviation

MS: maturity stage at harvest; S: storage days of whole fruits at 15 ºC and 90% RH; CI: colour index (*L*, *a*, and *b* from the Hunter Lab. Scale).

Table 2. Analysis of covariance for the effect of antioxidant treatment (AT), maturity stage (MS), storage (S) time of persimmons at 15ºC before processing, and their interactions on the colour parameters *L** and *a**, firmness, free radical scavenging activity, total phenolic content and total carotenoid content of fresh-cut 'Rojo Brillante' persimmons during storage at 5ºC.

	*L**	*a**	Firmness	Free radical scavenging activity	Total phenolic content	Total carotenoid content
AT	39.12**	26.33**	3,912.19**	2.99*	20.26**	0.24
MS	1,299.36**	3,977.92**	23.25**	46.95**	116.69**	236.28**
S	6.24*	0.81	6.30**	5.99*	8.72**	1.00
AT x MS	31.59**	46.70**	69.61**	0.60	5.19**	0.57
AT x S	4.44**	1.15	14.36**	1.44	8.10**	0.38
MS x S	4.58*	6.00*	0.58	50.61**	28.56**	2.69

Numerical values are the *F*-ratio of the variance.

* Significant *F*-ratios at $p \leq 0.05$

** Significant *F*-ratios at $p \leq 0.001$

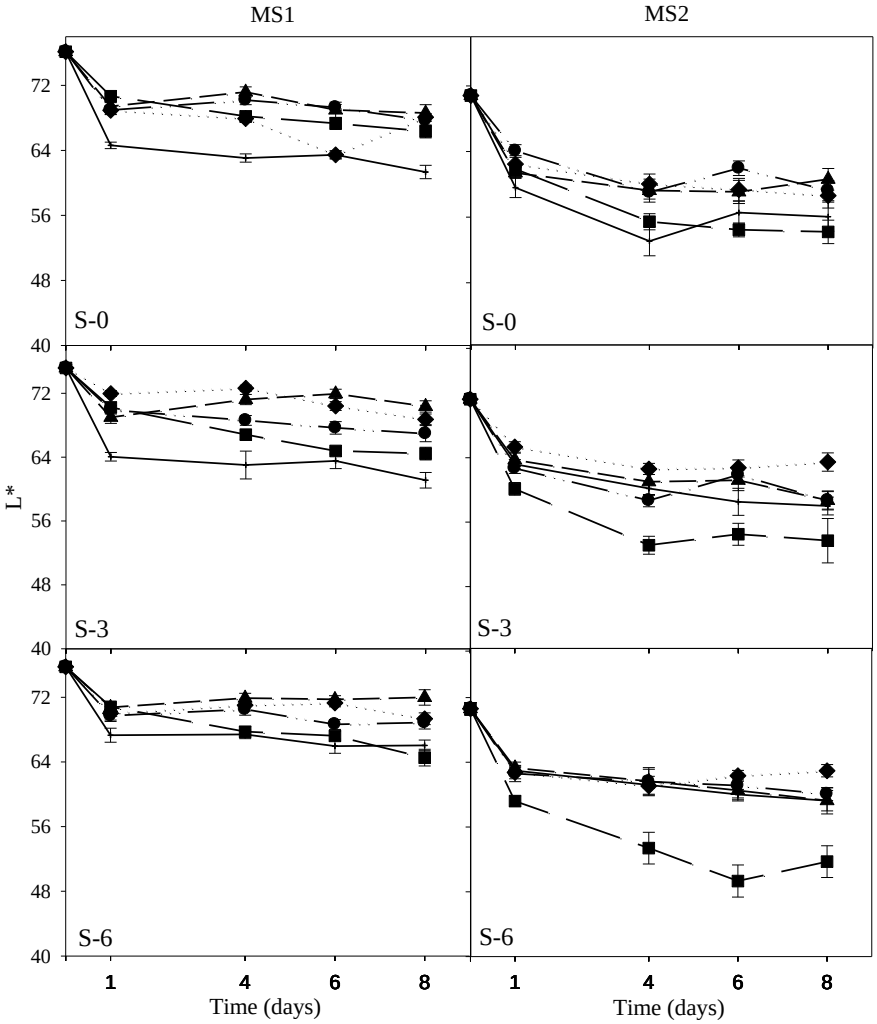

Fig. 1. Colour *L** changes of fresh-cut 'Rojo Brillante' persimmons dipped in 0.01 kg L^{-1} ascorbic acid (AA) ( ), 0.01 kg L^{-1} citric acid (CA) ( ), 0.005 kg L^{-1} cysteine (Cys) ( ), 0.005 kg L^{-1} $CaCl_2$ ( ) and water as a control ( ) during 8 days at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2) after 0, 3 and 6 days of storage at 15 ºC (S-0, S-3, and S-6). Shown data are mean ± standard error.

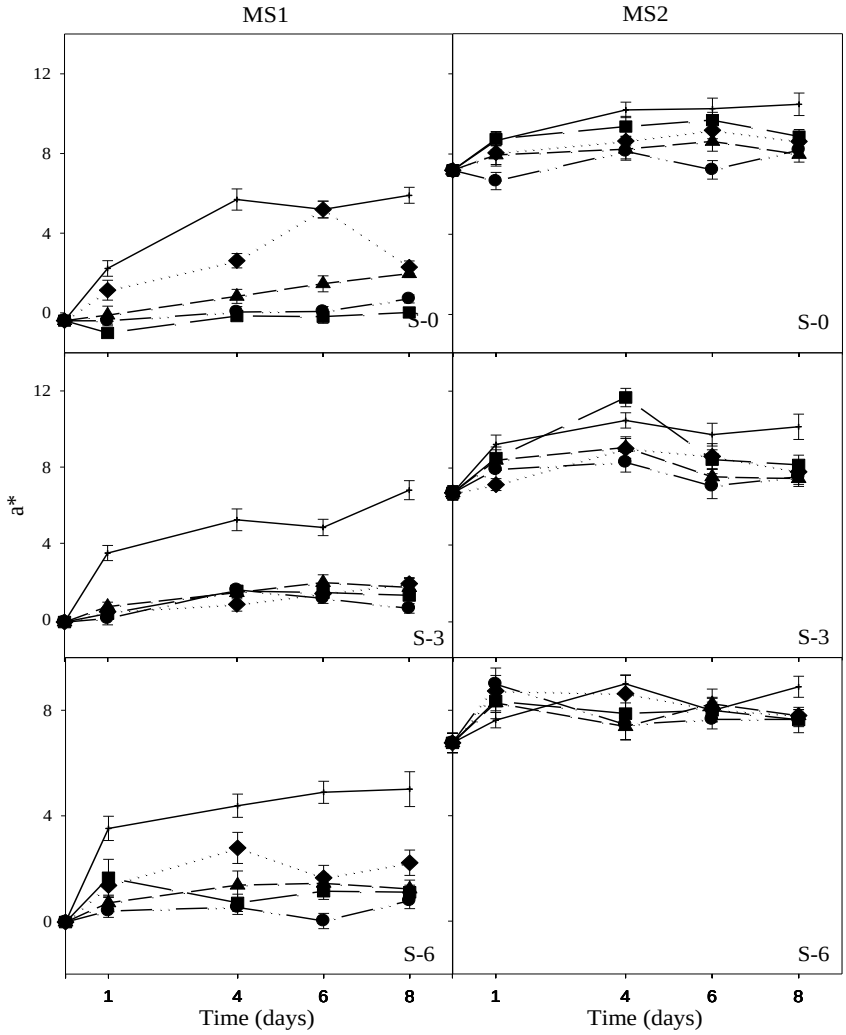

Fig. 2. Colour *a** changes of fresh-cut 'Rojo Brillante' persimmons dipped in 0.01 kg L^{-1} ascorbic acid (AA) (—▲—), 0.01 kg L^{-1} citric acid (CA) (—■—), 0.005 kg L^{-1} cysteine (Cys) (—▲—), 0.005 kg L^{-1} $CaCl_2$ (···◆···) and water as a control (—●—) during 8 days at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2) after 0, 3 and 6 days of storage at 15 ºC (S-0, S-3, and S-6). Shown data are mean ± standard error.

At MS2, the effectiveness of antioxidant treatments diminished as the storage time at 15 ºC before processing was prolonged (Figs. 1 and 2). Thus no significant differences in the a* values among treatments were found for the persimmons processed after the 6 days storage at 15 ºC (S-6). Furthermore, the CA application showed no significant differences as compared with the control samples in the a* values for those fruits processed after S-0 and S-3 days, and L* lowered significantly during storage.

From these results, it can be stated that antioxidant treatments were more effective for the persimmon fruits harvested at an earlier MS than those harvested at more advanced maturity. These differences in the effect of antioxidant treatments to control enzymatic browning may be due to changes in the phenolic content and PPO activity of fresh-cut persimmons at different MSs. Similar behaviour was reported in fresh-cut apples, where enzymatic browning was more efficiently controlled by antioxidants in mature-green and partially-ripe fruit than in ripe tissue (Soliva-Fortuny et al., 2002; Rojas-Graü et al., 2007). This result was attributed to chloroplast disintegration as maturity increases, which would caused the solubilisation of PPO and an increase in browning oversensitivity.

3.3. Fruit firmness

The antioxidant treatment, the MS at harvest, and the storage time at 15 ºC before processing fresh persimmons all had a significant effect on firmness (Table 2). Significant interactions were also observed between antioxidant treatments and the MS, and also between the MS and the storage time at 15 ºC before processing. In the persimmon fruits with MS1, the antioxidant treatments had a significant effect on firmness, while these differences were less marked at MS2 (Fig. 3). When the persimmon fruits with MS1 were processed after harvest (S-0), the Cys-treated and control samples showed greater firmness than the remaining treatments, including $CaCl_2$. As the processing day was delayed from S-3 to S-6, the differences between antioxidant treatments and the control were minimal, except for 0.01 kg L^{-1} CA, which always showed the least firmness during the storage at 5 ºC. Reduced fruit firmness by acid solutions has also been reported in some fruit tissues, which indicates

some damage of the cell wall structure. Thus in fresh-cut pears, the application of AA reduced firmness by up to 20%, whereas a 5% reduction has been reported in the control samples (Oms-Oliu et al., 2006). In fresh-cut apples at a ripe stage, N-acetylcysteine has been found to be more effective to maintain firmness than AA (Rojas-Graü et al., 2007). Although the role of calcium to reduce tissue softening is well-known, in our work $CaCl_2$ was not effective enough to maintain fresh-cut persimmon firmness. Some works have reported a relation between the MS of tissue and the effect of calcium treatments. Thus a dipping treatment containing $CaCl_2$ has been found to be effective to reduce softening in green and green-mature melons, but was unable to delay mature fruit softening (Oms-Oliu et al., 2007), and similar results in different pears cultivars have been reported (Dong et al., 2000; Soliva-Fortuny et al., 2004).

Firmness changes are supposed to be triggered by the action of pectin enzymes, especially polygalacturonases (Knee, 1973). During maturation, ripening and senescence, the rapid synthesis of these enzymes affects the fruit cell wall, and leads to the subsequent solubilisation and depolymerisation of pectic substances, and to the release of soluble Ca, which translates into a higher degree of softening (Soliva-Fortuny et al., 2002). In 'Rojo Brillante' persimmons, Salvador et al. (2007) used Cryo scanning electron microscopy to show the cell wall degradation of fruits as the MS advanced, which was correlated with loss of firmness. These authors showed substantial changes in the microstructure of persimmon fruits as the MS advanced. In particular, fruits with similar MSs to those studied herein (MS1 and MS2) showed degraded parenchyma tissue and a large amount of soluble solids, together with a certain amount of insoluble material, invaded the intracellular spaces. Loss of intracellular adhesion was also observed for late season persimmons (advanced MSs) by these authors. Therefore, this would probably explain not only the changes observed in 'Rojo Brillante' persimmon firmness as compared to the $CaCl_2$ treatment in our study, but also the fewer differences among antioxidant treatments as the MS at harvest and storage time before processing advanced.

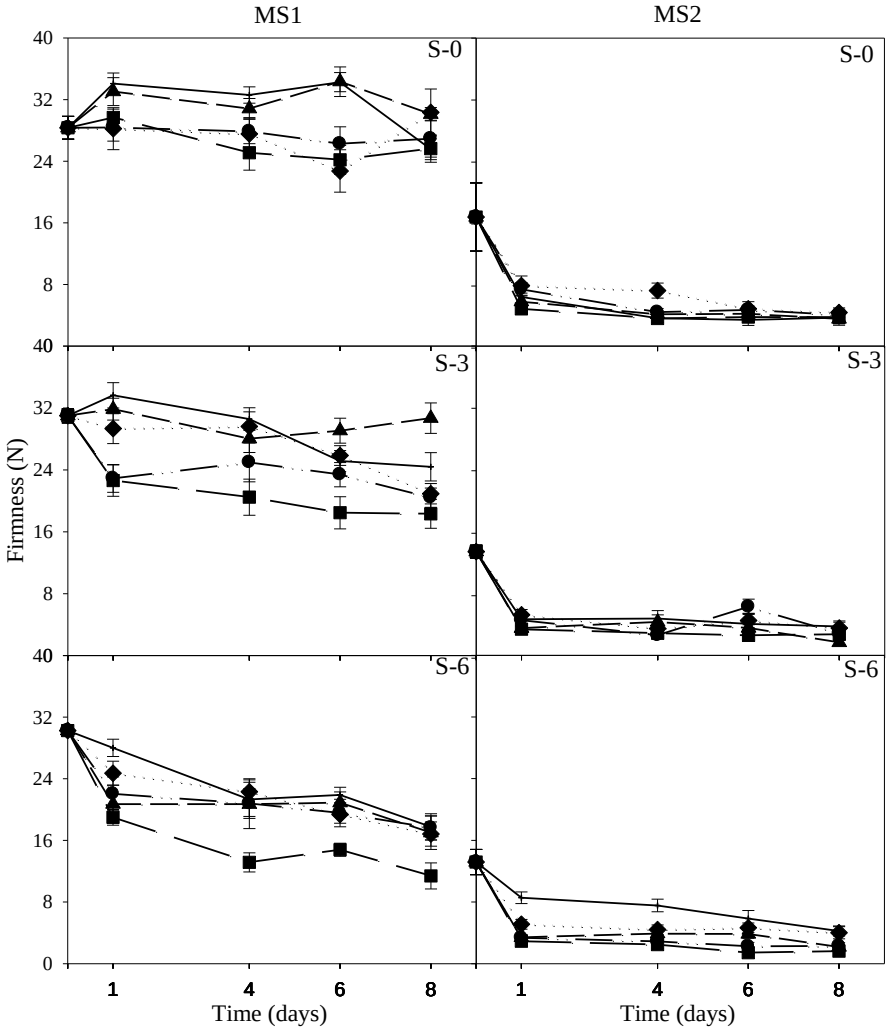

Fig. 3. Firmness of fresh-cut 'Rojo brillante' persimmons dipped in 0.01 kg L^{-1} ascorbic acid (AA) (—▲—), 0.01 kg L^{-1} citric acid (CA) (—■—), 0.005 kg L^{-1} cysteine (Cys) (—▲—), 0.005 kg L^{-1} $CaCl_2$ (···◆···) and water as a control (—●—) during 8 days at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2) after 0, 3 and 6 days of storage at 15 ºC (S-0, S-3, and S-6). Shown data are mean ± standard error.

3.4. Sensory quality

The visual appearance of persimmon slices, based on colour and general appearance, was assessed by a trained sensory panel (Fig. 4). In general, the persimmons processed after the different storage periods at 15 ºC followed the same trend. The control samples were always evaluated as poor or inedible after 1 day of processing, followed by $CaCl_2$ treatment, which reached the limit of marketability at day 1 after processing.

When persimmons were harvested at MS1 and directly processed (S-0), the application of AA or CA allowed a limit of marketability of 7-8 days of storage at 5 ºC. Yet this limit lowered to 6 days for the persimmon fruits processed after 3 and 6 days of storage at 15 ºC (S-3 and S-6). All the other antioxidant treatments came close to or were below the limit of marketability, even on day 1 of storage.

The persimmons processed at MS2 displayed a similar behaviour, although Cys was evaluated above the limit of marketability after 1 day of storage at 5 ºC for persimmons S-0 and S-3. Under these processing conditions, AA maintained persimmon slices within the limit of marketability over a storage period lasting 6-8 days, whereas CA only achieved 4 days of commercial shelf life at 5 ºC. For persimmons S-6, the limit of marketability was reached with AA after 6 days of storage at 5 ºC, as observed in the persimmons harvested at MS1. However, effectiveness was lost with CA in the persimmon fruits with this MS.

At the time of processing, 'Rojo Brillante' persimmons were evaluated to have an overall flavour of 7 and a firmness of 4, independently of the MS and the storage time at 15 ºC before processing (data not shown). After 8 days of storage at 5 ºC, the overall flavour of the persimmon slices remained within the range of acceptability (4-6) (Table 3). The application of 0.005 kg L^{-1} Cys induced a slight off-flavour in the samples, and samples were evaluated with the lowest flavour quality at the end of the 8 days storage. Richard-Forget et al. (1992) reported that the application of Cys is often incompatible with product taste due to the formation of sulphur compounds, as observed in the present work. The firmness evaluation confirmed the results obtained in the texture analysis. The processed samples with MS1 were evaluated

as firm (values above 3) after 8 days of storage at 5 ºC, whereas those with MS2 were evaluated as soft (values below 3).

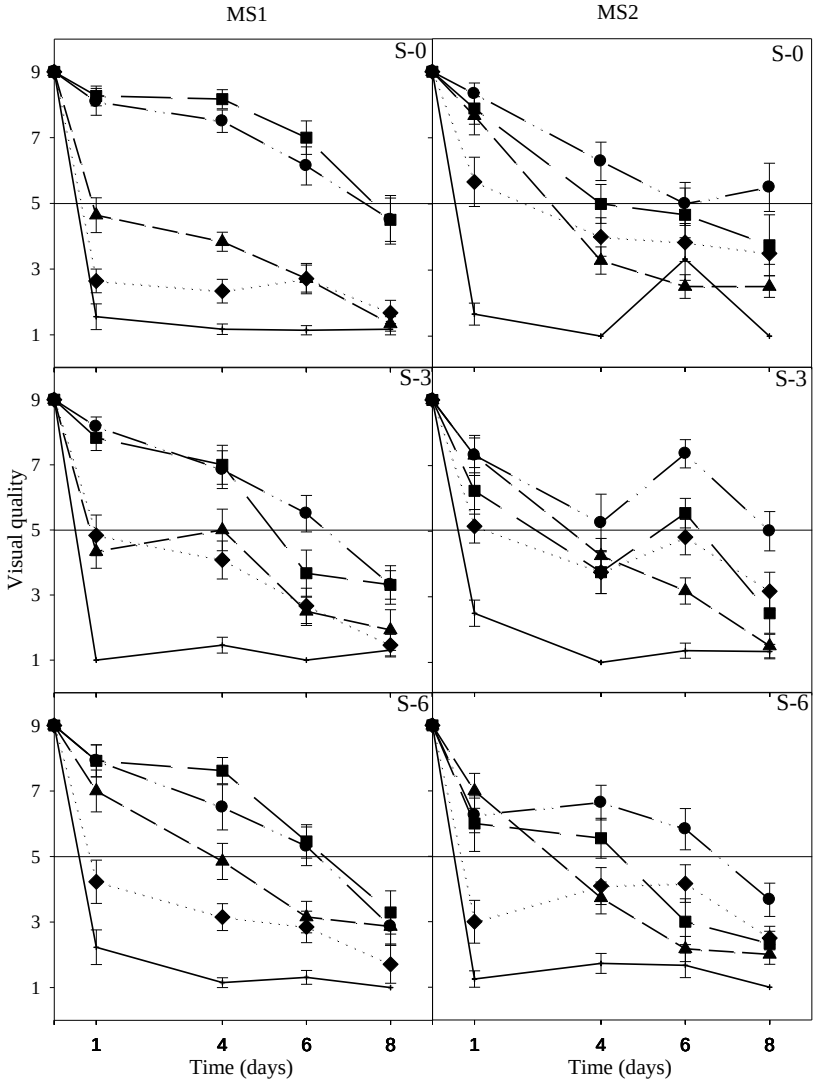

Fig. 4. Visual quality of fresh-cut 'Rojo brillante' persimmons dipped in 0.01 kg L-1 ascorbic acid (AA) ( ), 0.01 kg L-1 citric acid (CA) ( ), 0.005 kg L-1 cysteine (Cys) ( ), 0.005 kg L-1 CaCl2 ( ) and water as a control ( ) during 8 days storage at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2) after 0, 3 and 6 days of storage at 15 ºC (S-0, S-3, and S-6). Shown data are mean ± standard error.

Table 3. Effect of maturity stage (MS), storage (S) days at 15ºC before processing, and antioxidant treatment (AT) on the overall flavour, off-flavours and sensory firmness of fresh-cut 'Rojo Brillante' persimmons after 8 days of storage at 5ºC.

MS	AT	S-0			S-3			S-6		
		Overall flavour	Off-flavour	Firmness	Overall flavour	Off-flavour	Firmness	Overall flavour	Off-flavour	Firmness
1	0.01 kg L^{-1} AA	5.5 abA	0.2 bB	3.7 aA	5.7 aA	0.1 bA	3.4 abA	5.7 aA	0.2 aA	3.6 aA
	0.01 kg L^{-1} CA	5.7 aA	0.3 bB	3.6 aA	5.5 aA	0.2 bA	2.8 bA	5.1 aA	0.2 aA	3.0 bA
	0.005 kg L^{-1} Cys	4.1 bA	1.0 aA	3.8 aA	4.5 bA	1.0 aA	3.7 aA	5.5 aA	0.5 aA	3.1 abA
	0.5% kg L^{-1} $CaCl_2$	6.0 aA	0.2 bB	3.7 aA	5.6 aA	0.3 bA	3.3 abA	5.2 aA	0.2 aA	3.6 aA
	Control	5.0 abA	0.2 bB	4.0 aA	5.8 aA	0.2 bA	3.4 abB	5.5 aA	0.2 aA	3.3 abA
2	0.01 kg L^{-1} AA	5.3 abA	1.2 aA	2.6 aB	5.5 aA	0.3 bA	2.7 abB	6.0 aA	0.5 aA	2.9 aB
	0.01 kg L^{-1} CA	5.0 abA	1.3 aA	2.1 aB	5.4 aA	0.3 bA	2.3 bA	5.2 abA	0.5 aA	1.9 bB
	0.005 kg L^{-1} Cys	4.0 bA	0.8 aA	2.6 aB	3.4 bA	1.5 aA	3.0 aB	4.6 bB	0.6 aA	2.4 aB
	0.005 kg L^{-1} $CaCl_2$	5.3 abA	1.2 aA	2.6 aB	5.4 aA	0.5 bA	3.0 aA	5.9 aA	0.6 aA	2.5 aB
	Control	5.8 aA	1.3 aA	2.5 aB	5.4 aA	0.2 bA	2.8 abA	5.8 aA	0.4 aA	2.5 aB

For each maturity stage, small letters show significant differences among antioxidant treatments ($p \leq 0.05$). For similar treatments, capital letters show significant differences between maturity stages (MS) ($p \leq 0.05$).

AA: ascorbic acid; CA: citric acid; Cys: cysteine.

Overall flavour was rated on a 9-point scale, where 1: very poor and 9: excellent. Off-flavour was rated on a 5-point scale, where 1: no presence and 5: marked presence. Firmness was rated on a 5-point scale, where 1: very soft and 5: very firm.

3.5. Changes in free radical scavenging activity

Fig. 5 shows the free radical scavenging activity, expressed as the persimmon extract needed to reduce the $DPPH^{\bullet}$ (EC_{50}) by 50%; thus the lower the value, the higher the antiradical capacity of persimmon fruit. The radical scavenging activity of fresh-cut persimmon did not change during storage at 5 ºC. Therefore, data are provided for the samples after 8 days of storage at 5 ºC. In general, the antiradical capacity of Rojo Brillante persimmons increased as the MS and the storage time at 15 ºC before processing increased. In the fruit with MS1, the antiradical capacity increased by 50% as the storage time at 15 ºC advanced from S-0 to S-6. When comparing MSs, the initial free radical scavenging activity values increased by 60-70% as the MS advanced, except for the persimmons processed after 6 days of storage at 15 ºC (S-6). Although there were significant differences among antioxidant treatments, the results were variable and no conclusion as to treatment application can be drawn. The differences observed might be due to the natural heterogeneity of fruit.

Persimmon fruits are characterised by a high antioxidant capacity given by a high free radical scavenging capacity (de Ancos et al., 2000; Chen et al., 2008). Although no data have been reported on the effect of the MS and postharvest storage on the antioxidant capacity of persimmon fruit, several authors have reported variations in the antioxidant capacity in plants induced by abiotic stress conditions. Prior et al. (1998) found increased antioxidant capacity, total phenols and anthocyanins with more advanced maturity at harvest in a wide variety of berries. The same results have been observed in peppers, apricots and cucumbers (Hegedüs et al., 2011; Navarro et al., 2006; Sudha et al., 2011).

3.6. Total phenolic content (TPC)

TPC was significantly influenced by the MS, the storage time at 15 ºC before processing and antioxidant treatments (Fig. 5). The initial TPC values of the persimmons processed at MS1 after 0 and 3 days of storage at 15 ºC (S-0 and S-3) were around 8 mg gallic acid/100 g of sample, whereas those of the samples processed after 6 days stored at 15 ºC (S-6) were around 20 mg gallic acid/100 g of sample. At MS2, the initial TPC of the persimmon fruit ranged between 7 and 12 mg gallic acid/100 g of

sample. The differences observed among these values could be attributed more to biological variation than to an effect of the MS at harvest and postharvest storage period at 15 ºC. Some works have reported lower TPC with fruit ripening in banana (Ibrahim et al., 1994), guava (Bashir and Abu-Goukh, 2003) or strawberry (Ferreyra et al., 2007).

TPC of fresh-cut persimmon was not affected by storage at 5 ºC. After cutting and storage at 5 ºC, TPC generally increased to values of between 12 and 16 mg gallic acid/100 g of sample, which in some cases represented an increase of 60% from the initial values. These results indicate the influence of processing on phenolic compounds. Reyes and Cisneros-Zevallos (2003) reported that potato wounding induced the synthesis of phenolic compounds, which translated in higher TPC values. However, no differences have been reported in TPC between whole and fresh-cut kiwifruit, watermelon or pineapple (Gil et al., 2006).
Although some significant differences were found among antioxidant treatments, the results were variable, which means that it is impossible to conclude the effectiveness of treatments on TPC in fresh-cut persimmons. Some works have reported the effect of AA and CA on maintaining the TPC of fresh-cut fruits. For example, the combination of AA and CA maintained the TPC of fresh-cut apple and mango during 8 and 12 days at 4 ºC, respectively (Cocci et al., 2006; Siddiq et al., 2013).

Considering the large contribution of phenolic compounds to the antioxidant capacity of fruits and vegetables, Fu et al. (2011) evaluated the correlation between antioxidant activity and TPC of 62 fruits. In their work, a high correlation was observed when total antioxidant capacity was evaluated as ferric-reducing antioxidant power, which indicated that phenolic compounds could be one of the main components responsible for the reducing antioxidant capacity of these fruits. However, a very weak correlation was reported when the trolox equivalent antioxidant capacity value was used, which suggests that phenolic compounds may not be the main components responsible for the free radical scavenging ability of these fruits. In our work, free radical scavenging capacity increased as the MS advanced from MS1 to MS2, and also after processing (cutting and storage at 5 ºC) in persimmon fruits harvested at MS1. Despite the TPC of 'Rojo Brillante' persimmons increasing after

processing, the results did not show a higher TPC value for fresh-cut persimmons as the MS at harvest advanced.

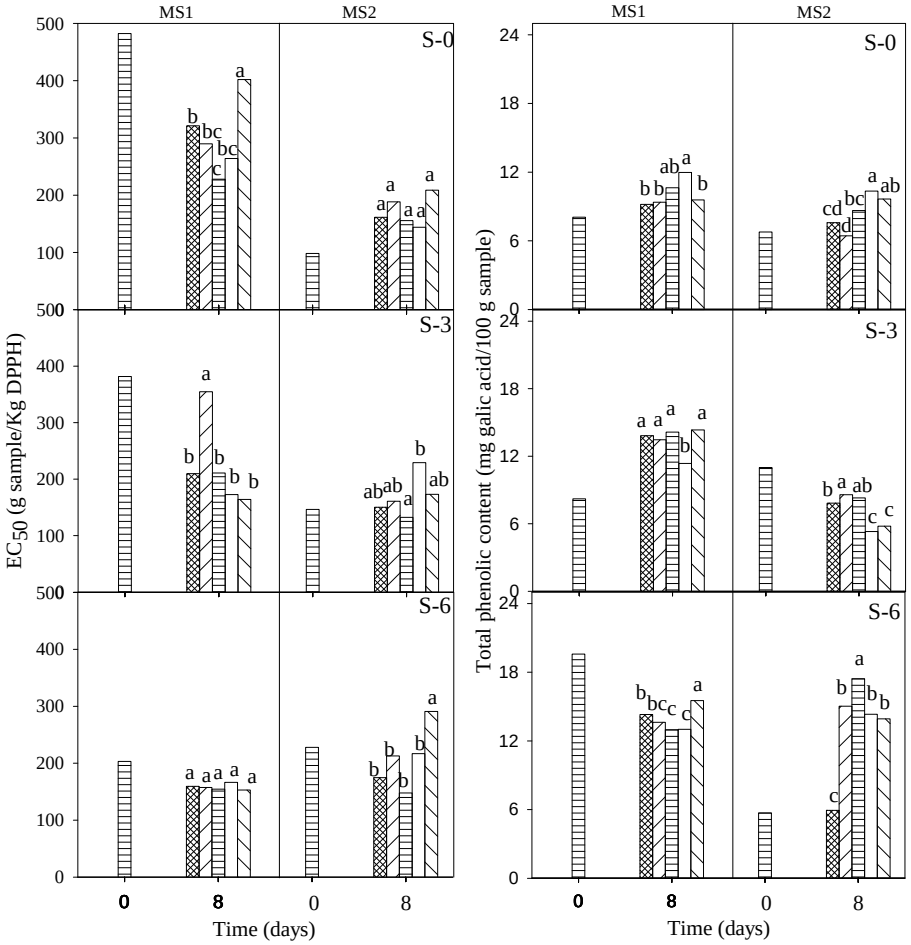

Fig. 5. Free radical scavenging activity expressed as EC_{50} and total phenolic content of fresh-cut 'Rojo brillante' persimmons dipped into 0.01 kg L^{-1} ascorbic acid (AA) (▩), 0.01 kg L^{-1} citric acid (CA) (▨), 0.005 kg L^{-1} cysteine (Cys) (▤), 0.005 kg L^{-1} $CaCl_2$ (▭) and water as a control (▧) over 8 days at 5 ºC. The results are average values. For each storage time at 5 ºC, bars with different letter are significantly different at the 95% level.

3.7. Carotenoids

In this work, eight carotenoids were identified in 'Rojo Brillante' persimmons: α-carotene, β-carotene and six xantophylles (trans-viloaxanthin, cis-violaxanthin, lutein, zeaxanthin, α-criptoxanthin and β-criptoxanthin). Table 4 shows the average values of the individual major carotenoids (α-carotene, β-carotene, α-criptoxanthin and β-criptoxanthin) and the provitamin A value of the fresh-cut 'Rojo Brillante' persimmons processed at the two MSs after different storage periods at 15 ºC.

Carotenoids of fresh-cut persimmons were not affected by antioxidant treatment, nor by storage at 5 ºC ($p>0.05$). Similar results have been previously found in Fuyu persimmon slices, fresh-cut papayas (Wright and Kader 1997; Rivera-López et al., 2005), kiwifruits and strawberries (Gil et al., 2006). The storage time at 15 ºC before processing had no effect on the major carotenoids in the fruits harvested at MS1, whereas an increase in the fruits harvested at MS2 was seen as the storage time at 15 ºC before processing increased. In general, the more advanced the MS stage, the higher the total carotenoid concentration. β-criptoxanthin and β-carotene were the carotenoids with the highest values, and represented around 48-50% and 27-30%, respectively, in the persimmons processed at MS1, and around 56-67% and 18-31% in those processed at MS2. Other works have indicated that lycopene, together with β-cryptoxanthin and β-carotene, are the main carotenoids in 'Rojo Brillante' persimmons (de Ancos et al., 2000; Plaza et al., 2012). The high lycopene content reported in 'Rojo Brillante' persimmon by other authors can be attributed to a more advanced MS of fruit.

The high β-criptoxanthin content in Rojo Brillante persimmons contributed largely to the provitamin A value. Considering that the recommended daily allowance (RDA) for females is 800 RE, the persimmons harvested at MS2 provided between 5 and 8% of the RDA in a 100 g serving. This contribution was larger than those reported in the literature for other persimmon cultivars (Giordani et al., 2011). These differences can be explained by the extraction method, cultivar, fruit variability or the different fruit MSs employed.

Table 4. Effect of maturity stage (MS) and storage (S) days at 15ºC before processing on the concentration (μg/100g sample) of the major carotenoids and retinol equivalent (RE) of fresh-cut 'Rojo Brillante' persimmons. The results are average values of all the treatments (antioxidants and control) ± standard deviations.

	MS1						MS2					
	S-0		S-3		S-6		S-0		S-3		S-6	
α-Criptoxanthin	49.7 ± 4.3	aA	58.3 ± 4.6	aA	50.9 ± 6.0	aB	45.8 ± 4.0	cA	67.2 ± 4.9	bA	98.9 ± 10.9	aA
β-Criptoxanthin	139.0 ± 7.3	aB	144.3 ± 4.7	aB	138.1 ± 8.1	aB	239.4 ± 12.5	bA	402.1 ± 12.4	aA	477.1 ± 14.6	aA
α –Carotene	9.2 ± 0.8	aA	9.0 ± 1.3	aB	10.5 ± 3.7	aA	11.5 ± 1.8	bA	24.2 ± 2.6	aA	16.7 ± 1.6	bA
β –Carotene	87.7 ± 3.4	aB	94.2 ± 4.5	aA	75.4 ± 4.0	bB	128.3 ± 10.9	abA	112.9 ± 11.0	aA	147.9 ± 9.7	bA
Total	285.7 ± 14.1	abB	305.8 ± 16.4	aB	274.8 ± 12.6	bB	424.9 ± 12.4	bA	606.4 ± 14.0	bA	740.6 ± 14.8	aA
RE	25.8 ± 1.1	abB	27.2 ± 1.2	aB	3.6 ± 0.9	bB	42.2 ± 3.2	bA	51.0 ± 3.2	bA	65.7 ± 4.9	aA

For each maturity stage, small letters indicate significant differences among storage days at 15ºC before processing (S-0, S-3, S-6). For similar storage periods at 15ºC before processing, capital letters indicate significant differences between maturity stages (MS1, MS2).

3. Conclusion

The results indicate that the selection of an adequate MS at harvest before processing is a determinant for the commercial shelf life of fresh-cut 'Rojo Brillante' persimmons. The use of acidulants, such as AA or CA, controls tissue browning and maintains the general visual quality of fresh-cut persimmons above the limit of marketability by up to 6-8 days of storage at 5 ºC in those fruits harvested at an earlier MS, whereas CA is less effective than AA for late-season persimmons. Free radical scavenging activity, TPC and carotenoid content were not negatively affected by cutting and the dipping treatment, whereas free radical scavenging activity and total carotenoid content increased in late-season persimmons. For commercial purposes, the 'Rojo Brillante' persimmons harvested at MS1 can be processed as a fresh-cut commodity, even after 3 days of storage at 15 ºC if treated with AA or CA. However, processing the fruits with MS2 after harvest and being treated with AA are recommended.

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Chapter 2

Physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmon affected by maturity stage and antibrowning agents

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ABSTRACT

To prevent enzymatic browning of fresh-cut 'Rojo Brillante' persimmon, different combinations of ascorbic acid (AA) and citric acid (CA) with calcium chloride ($CaCl_2$) were tested in fruit harvested at two maturity stages (MS1 and MS2). Color, firmness, sensory quality, total vitamin C, radical scavenging activity, total phenolic content and carotenoids were evaluated over 9 days of storage at 5 ºC. Antioxidant dips reduced enzymatic browning if compared to the control samples. Selecting fruits with good firmness and the addition of 10 g L^{-1} $CaCl_2$ help prevent loss of the firmness of fresh-cut 'Rojo Brillante' persimmons treated with acidic solutions as antioxidants to control enzymatic browning. The limit of marketability of the persimmon fruit processed at MS1 was significantly reduced by the burst of the disorder known as 'flesh browning', and only the samples treated with 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ maintained a limit of marketability close to 7 days. At MS2, all the antioxidant solutions allowed a limit of marketability of 7 storage days at 5 ºC. Nutritional quality was not affected by either antioxidant dips or cutting processes, but MS at harvest was.

Keywords: Antioxidants, minimally processed, radical scavenging activity, total vitamin C, total phenolic content, carotenoids.

INTRODUCTION

Persimmon fruits have become an important alternative crop to citrus fruit in the Mediterranean region of Spain due to the expansion of the cultivar 'Rojo Brillante', which is greatly appreciated by consumers for its color, size, flavor and nutritional value. When harvested, it is an astringent variety, but the application of high CO_2 levels allows the elimination of astringency, while preserving fruit quality and firmness (Arnal and del Río, 2003). This technology has opened up new national and export markets, and makes this cultivar a good candidate to be commercialized as a minimally processed fruit. However, physical damage during the peeling and cutting processes leads to major physicochemical changes that reduce the product's shelf life, being enzymatic browning and softening the main causes of quality loss (Sanchís et al., 2015). In recent works conducted in fresh-cut 'Rojo

Brillante' persimmon, concentrations of 10 g L^{-1} ascorbic acid (AA) or 10 g L^{-1} citric acid (CA) controlled tissue browning and maintained the visual quality of fresh-cut persimmon above the limit of marketability for 6-8 storage days at 5 ºC, depending on the maturity stage (Ghidelli et al., 2013; Sanchís et al., 2015). However, these acidic solutions reduced fruit firmness as compared to control samples (Sanchís et al., 2015). The same studies also showed some antioxidant activity for 5 g L^{-1} $CaCl_2$ dips. However, this treatment had a limited effect to preserve fresh-cut persimmon from texture loss, probably because calcium levels were too low (Sanchís et al., 2015). Several works have described a synergic effect to control enzymatic browning and to reduce firmness loss of fresh-cut fruits when antioxidants are combined with $CaCl_2$. For example, Chiabrando and Giacalone (2012) found that the application of 10 g L^{-1} of CA/$CaCl_2$ or AA/$CaCl_2$ effectively controlled enzymatic browning in different cultivars of apples slices. The same results were obtained by Tortoe et al. (2007) when a mixture of AA and $CaCl_2$ was applied to apple cylinders.

On the other hand, although appearance, texture and flavor are the main properties that determine the quality value of fresh-cut products at the time of purchase, nutritional quality is also an important driving force behind the increased interest of consumers for fresh-cut fruits (Kader, 2002). Persimmon fruits are generally recognized as an outstanding source of biologically active compounds such as carotenoid and other phenolic compounds that contribute to their antioxidant and free-radical scavenging properties (Giordani et al., 2011). The effect of processing persimmon fruits on bioactive compounds has been scarcely reported. These studies include the effect of controlled atmosphere storage in vitamin C and carotenoid content of fresh-cut 'Fuyu' persimmons (Wright and Kader, 1997a, b) and antioxidant dips and maturity stage in free radical scavenging activity, phenolic content and carotenoid content of 'Rojo Brillante' persimmons (Sanchís et al., 2015). In this framework, the aim of this work was to evaluate the effect of the combination of AA or CA with $CaCl_2$ on the physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmon harvested at two maturity stages (MS), as this combinations have never been studied to extend its shelf life.

MATERIALS AND METHODS

Reagents and solvents

The antibrowning agents tested, ascorbic acid (AA) and citric acid (CA), were supplied from Quimivita (Barcelona, Spain). Calcium chloride ($CaCl_2$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, sodium chloride, disodium hydrogen phosphate, ammonium acetate, 4-methylcathecol, polyvinylpolypyrrolidone, β-apo-8'-carotenal, and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, chlorhydric acid, ethanol, hexane, methylene chloride, acetonitrile, and butylated hidroxytoluene were acquired from Scharlau (Barcelona, Spain). β-cryptoxanthin, lutein, lycopene, and zeaxanthin were provided by Extrasynthese (Genay, France). Gallic acid came from Acros Organics (Geel, Belgium) and thriethylamine from Panreac (Barcelona, Spain). All the solvents used were of HPLC-grade and Milli-Q system ultra-pure water (Millipore Corp., USA) was used throughout.

Plant material and sample preparation

Persimmon fruits (*Diospyros kaki* cv. Rojo Brillante) were provided by a local packinghouse assigned to the persimmon geographical indication 'Denominación de Origen Kaki Ribera del Xúquer' (Valencia, Spain) at two different commercial maturity stages (MSs), determined by external color as MS1 with a color index (CI) of -0.86 and MS2 with a CI of 11.95, where CI=1,000*a/L*b. Natural astringency of 'Rojo Brillante' persimmons was eliminated by placing them in closed chambers with an atmosphere containing 95±2% CO_2 at 20 ºC and 90% RH. The physicochemical characteristics of persimmon fruits before processing were evaluated in 30 fruits for soluble solids content (Atago Pocket refractometer, Atago company Ltd., Japan), total acidity (Titration Excellence T50, Mettler Toledo, Barcelona, Spain), external color (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Osaka, Japan), and firmness (Instron Universal Machine, Model 3343, Instron, Barcelona, Spain). External color, expressed as CI, was calculated using the Hunter L, a, b color space, and fruit firmness was the maximum force

expressed in newtons (N) required to penetrate fruit flesh after removing skin on the equator.

Persimmons were washed, peeled and cut into eight wedges. Pieces were dipped into the antioxidant solutions or water as control for 3 min, and were allowed to drain and dry under cold conditions. The tested antioxidant solutions were: 10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$ (w/v), 5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$, 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$. Once dried, four pieces (115±10 g) were placed on polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and were heat-sealed with microperforated films (35-µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure that the atmosphere on the tray was not modified, the polypropylene film was also perforated with a needle (four perforations, 1 mm in diameter). Finally, samples were stored up to 9 days at 5±1 ºC. A total of 6 trays per treatment and sampling time were prepared that corresponded to 3 trays for physico-chemical and sensory analysis and 3 trays for nutritional analysis. For nutritional quality, samples were frozen in liquid nitrogen and stored at -80 ºC until analysis.

Color and firmness measurements

The color of the persimmons pieces was determined with a Minolta CR-400 chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) on 12 pieces per treatment using CIELAB color parameters L* and a*. Each measurement was taken randomly at 3 locations per sample piece. A standard white calibration plate was employed to calibrate the equipment. The results are expressed as the means of 12 samples per treatment.

Firmness of fresh-cut persimmons 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 at a depth of 2 mm and at a speed of 5 mm/s. Twelve samples per treatment were measured and the results were expressed in newtons (N).

Sensory quality

The sensory quality of persimmon slices was conducted by 15 trained judges, and included a visual and taste evaluation. 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 color photograph of the samples rated by this scale was used. Each treatment was presented to the panelists on trays, which held 12 persimmon pieces, labeled with a 3-digit random code, to account for sample variability.

The taste evaluation included off-flavor, firmness and the overall flavor of the fresh-cut 'Rojo Brillante' persimmons. Off-flavor 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 flavor was rated on a 9-point scale, where 1-3 represented a poor quality range, 4-6 an acceptable quality range, and 7-9 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, were presented to the panelists on the trays labeled 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 color, samples were illuminated with appropriate lighting to completely mask browning.

Bioactive compounds

Total Vitamin C. Total vitamin C was determined as the sum of ascorbic acid and L-dehydroascorbic acid as described by Wright and Kader (1997a). Two grams of persimmon samples, which had been stored at –80 ºC, were homogenized with 38 mL of a solution of 0.1 M citric acid and 0.05% ethylenediaminetetraacetic acid in 5% aqueous methanol for 2 min at 22000 rpm (Ultraturrax, IKA, Germany). Two mg of D-isoascorbic acid were added as an internal standard. The homogenate was centrifuged at 10000 rpm for 5 min at 4 ºC. Next 1.5 mL of supernatant was reacted with 0.5 mL of 1,2-phenylenediamine (3.33 mg/mL) diluted in methanol:water (5:95, v/v). The mix was kept for 37 min in the dark at room temperature. Afterward samples were passed through a 0.45 μm membrane filter into an amber vial and sealed for their analysis by high pressure liquid chromatography (HPLC). The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300), and diode array detector (Model

L-2450). A reversed-phase C18 LiChroCart® column (250 x 4 mm, 5 μm-particle, Merck, Darmstadt, Germany), preceded by a precolumn (4 x 4 mm), was used. The injection volume was 40 μL and the oven temperature was 4 ºC. The mobile phase was a solution of methanol:water (5:95, v/v) that contained 5 mM of hexadecyltrimethylammonium bromide and 50 mM of ammonium dihydrogen phosphate, adjusted to pH 4.6. The flow rate was fixed at 1 mL/min. Ascorbic acid and D-isoascorbic acid were detected at 261 nm, whereas L-dehydroascorbic acid was detected at 348 nm. Total vitamin C *was expressed as mg of total vitamin C per 100 g sample.* Three replicates per treatment were determined.

Free radical scavenging activity. The free radical scavenging activity was determined by the method of Brand-Williams et al. (1995) using DPPH• as the free radical. Extraction was done as described by Chen et al. (2008), with some modifications. Two grams of persimmon pulp were mixed with 30 mL of 80% methanol. The solution was homogenized at 20000 rpm for 2 min (Ultraturrax, IKA, Germany), followed by boiling in a water bath for 20 min to inactivate the PPO enzyme. The homogenate was immersed in an ultrasonic machine at room temperature for 15 min and centrifuged at 10000 rpm for 20 min at 5 ºC. The resultant supernatant was then filtered and used as the persimmon extract. A second pulp extraction was necessary to complete the extraction. The mix of both extracts was used to analyze the antiradical capacity of the samples. Five methanolic dilutions from the supernatant were prepared to relate the decrease in $DPPH^{\bullet}$ absorbance with sample concentration. Seventy five μL of extract were mixed with 225 μL of $DPPH^{\bullet}$ (24 ppm). The mixture was kept in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 520 nm using a multiplate spectrum (Multiskan Spectrum, Thermo Fisher Scientific, Finland). $DPPH^{\bullet}$ radical scavenging activity was expressed as the effective concentration (EC_{50}). This value expresses the amount of persimmon extract required to reduce the initial $DPPH^{\bullet}$ concentration by 50%; thus, lower EC_{50} values mean greater antiradical capacity. Radical scavenging activity was expressed as g of persimmon fruit per Kg of $DPPH^{\bullet}$. Three replicates per treatment were determined.

Total phenolic content. Total phenolic content was measured following the method described by Chen et al. (2008). One gram of frozen sample was mixed with 15 mL of methanol with 1% hydrochloric acid. The mix was homogenized at 10000 rpm for 1 min (Ultraturrax, IKA, Germany), immersed in an ultrasonic bath for 30 min and centrifuged at 12000 rpm for 20 min at 4 ºC. The supernatant was filtered and collected. Extraction was repeated and the supernatants were combined for the analysis. Two methanolic dilutions were prepared with the extracts. Next 300 μL of supernatant were mixed with 600 μL of Folin Ciocalteu reagent and 2.4 mL of sodium carbonate solution (200 mg mL^{-1}) in this order. The mixture was incubated for 1 h in the dark at room temperature. The absorbance of the resulting solution was measured at 765 nm using a spectrum multiplate reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The results were expressed as mg of gallic acid for 100 g of persimmon fruit. Three replicates per treatment were determined.

Carotenoids. The extraction, saponification and quantification methods were performed as described by Wright and Kader (1997b). For the extraction, 5 g of sample were added to a centrifuge tube together with 10 mL of cold ethanol to be homogenized (Ultraturrax, IKA, Germany) for 3 min at 16000 rpm. Eight mL of hexane were added and the sample was homogenized for another 2 min. The mixture was then centrifuged for 4 min at 5000 rpm and 4 ºC. The organic phase was transferred to a 250-mL screw-cap Erlenmeyer flask. The extraction was repeated with 5 mL of saturated sodium chloride and 8 mL of hexane. The resultant organic phase was transferred to the Erlenmeyer flask with the first extract. For saponification, 15 mL of 10% methanolic potassium hydroxide were added to the Erlenmeyer flask. The flask was flushed with nitrogen, sealed, covered with aluminum foil to prevent oxygen and light, and left at room temperature for 16 h with gentle shaking. Next the mixture was transferred to a separatory funnel to remove potassium hydroxide with 15 mL of 10% sodium chloride, followed by deionized water until the mixture reached a neutral pH. The final extract was evaporated under nitrogen until dryness and was kept at -80 ºC until analyzed. At the time of the analysis, samples were redissolved in 200 μL of methylene chloride and 1.8 mL of the mobile phase. Major carotenoids

were determined by HPLC in three replicates per treatment. For the analysis, the resuspended sample (1.5 mL) was filtered though a 0.45 μm nylon filter into amber vials. The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), a quaternary pump (Model L-2130), a column oven (Model L-2300) and a diode array detector (Model L-2450). A reversed-phase C30 YMC-Pack column (250 x 4.6 mm, 5 μm-particle, Merck, Darmstadt, Germany) was used. The injection volume was 60 μL and the oven temperature was 4 ºC. The mobile phase consisted in acetonitrile, methanol containing 0.05 M ammonium acetate, and methylene chloride 75:20:5 (v/v/v) containing 0.1% butylated hydroxytoluene and 0.05% triethylamine. The flow rate was 1.5 mL/min. Detection occurred at 450 nm. Identification of peaks was confirmed using standards of major compounds. The results were expressed as μg per 100 g of fresh weight (FW). The retinol equivalent (RE) was calculated on the basis of 1 RE = 6 μg of β-carotene or 12 μg of other provitamin A carotenoids (β-cryptoxanthin and α-carotene).

Statistical analysis

Statistical analyses were performed by STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Any specific differences among treatments were determined by least significant differences (LSD) when the analysis of variance (ANOVA) showed a significant *F*-value. Significant differences were defined at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Physicochemical properties of whole persimmon fruit

Persimmon cv. 'Rojo Brillante' has a short maturation period, between October and December. External persimmon color is the property used as a nondestructive index for harvesting, whereas fruit firmness is the most important property to rate 'Rojo Brillante' persimmon quality when marketed after astringency has been removed (Salvador et al., 2007). In this work, fruits were harvested at two different ripening stages of commercial maturity, which corresponded at the beginning of October (MS1) and mid-November (MS2). At MS1 the

CI of the fruit corresponded to a yellow-orange color (-0.86±1.69) and intensified to orange-red (11.95±2.62) for MS2. These MSs were also differentiated by fruit firmness with 64.8±3.5 N for MS1 and 45.2±7.3 N for MS2. However, no significant differences were found between MSs in the soluble solids content and total acidity of persimmon fruit, and the values were 14.85±0.14 ºBrix and 1.13±0.16 g malic acid L^{-1}, respectively.

Color and Firmness of Fresh-Cut Persimmon

The browning of fresh-cut persimmon was accompanied by a decrease in the L* and an increase in the a* values as storage time at 5 ºC increased (Figure 1). At the time of processing, the persimmons slices with MS2 obtained lower and higher L* and a* values, respectively, than those with MS1. In both MSs, the control samples presented lower L* and higher a* values than the antioxidant-treated samples, and showed a beneficial effect of the antioxidant treatments to control enzymatic browning in fresh-cut persimmon. In a previous work conducted with fresh-cut 'Rojo Brillante' persimmon, some activity was seen with 5 g L^{-1} $CaCl_2$ as enzymatic browning was reduced, although the most effective antioxidants were 10 g L^{-1} AA and 10 g L^{-1} CA (Sanchís et al., 2015). In the same work, 10 g L^{-1} CA lost effectiveness in the fruits harvested in the second half of the season (MS2, CI of 7.6±1.7) if compared to those harvested earlier (MS1, CI of 1.5±1.0), and no differences were found in the a* values between the control and CA-treated samples at MS2. In the present work, the combination of $CaCl_2$ with AA or CA controlled enzymatic browning in both MSs, and no differences were observed among treatments for neither the L* nor the a* values. Inhibition of browning was not influenced by concentration of CA (5 g L^{-1} or 10 g L^{-1}) or MS at harvest, which was probably due to the combined effect of $CaCl_2$ with the antioxidant. Other studies have shown the effectiveness of AA or CA mixed with $CaCl_2$ to extend the shelf life of fresh-cut apples (Rocha et al., 1998) or pears (Soliva-Fortuny et al., 2004). However, the effectiveness of treatments depended on not only the cultivar, but also the maturity stage at harvest.

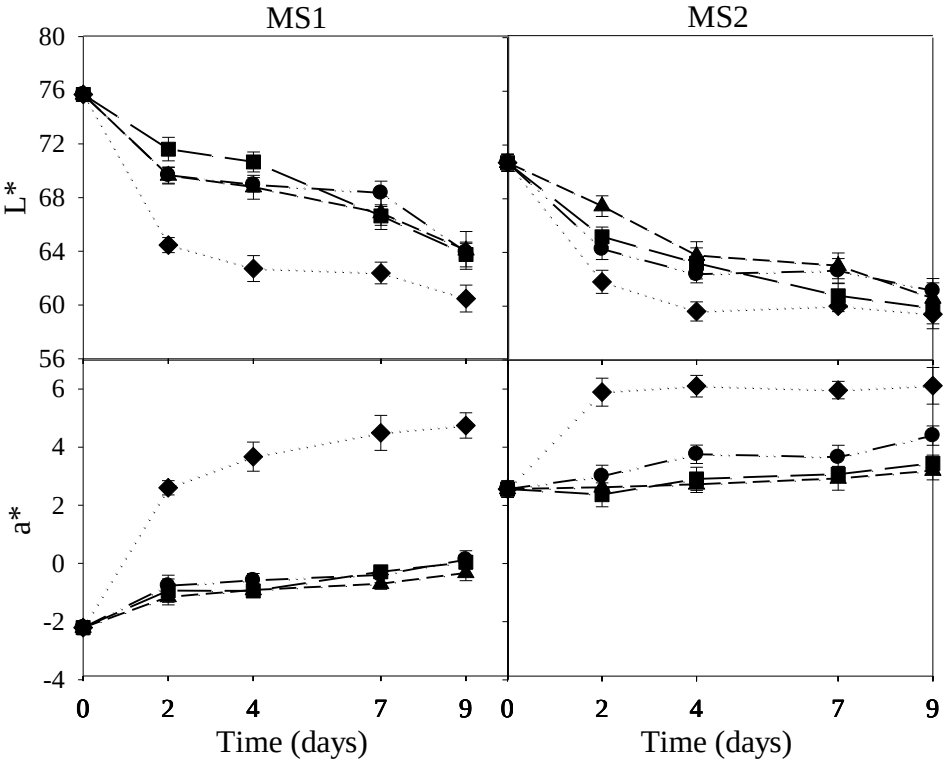

Figure 1. Color L* and a* changes in fresh-cut 'Rojo Brillante' persimmons dipped in 10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$ (—●—), 5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (—■—), 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (– –▲– –), and water as control (····◆····) for 9 days at 5 ºc. Persimmons were processed at two maturity stages (MS1 and MS2). Vertical bars are standard errors (n=12).

A high degree of firmness at harvest seems crucial to maintain good firmness after processing and storage at 5 ºC. Thus, the persimmons processed with MS1 maintained an average firmness after 9 storage days of 55±6 N, which represented a firmness loss close to 15%, whereas the fruits with MS2 displayed a firmness loss close to 45% (Figure 2). In general, the differences in firmness among treatments were minimal for both MSs. In a previous work, 5 g L^{-1} $CaCl_2$ did not prove sufficiently effective to maintain the firmness of fresh-cut 'Rojo Brillante' persimmons, the application of acidic solutions (10 g L^{-1} AA or 10 g L^{-1} CA) led to major tissue softening if compared to the control samples, and

the 10 g L^{-1} CA dips exerted the strongest effect to reduce firmness (Sanchís et al., 2015). In the present work, the combination of either CA (5 or 10 g L^{-1}) or 10 g L^{-1} AA with 10 g L^{-1} $CaCl_2$ maintained the firmness of the persimmon slices within the same range as the control samples during the storage for both MSs. Therefore, the present results indicate the beneficial effect of 10 g L^{-1} $CaCl_2$ to prevent firmness loss of fresh-cut persimmons treated with acidic solutions, used as antioxidants to control enzymatic browning.

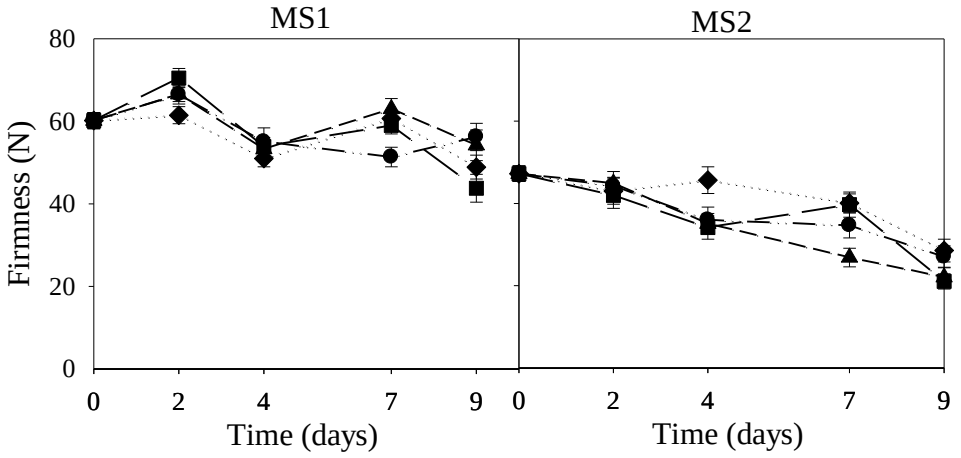

Figure 2. Firmness of fresh-cut 'Rojo Brillante' persimmons dipped in 10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$ (—●—), 5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (—■—), 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (– –▲– –), and water as control (·····◆·····) 9 days at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2). Vertical bars are standard error (n=12).

Sensory quality

The visual quality, based on color and general appearance, of persimmon slices was assessed by a trained sensory panel (Figure 3). The control samples were always evaluated as poor or inedible after 1 day of processing due to enzymatic browning. In the antioxidant-treated samples, the limit of marketability was reached by day 7 for the samples processed with MS2, but this period was shorter for the samples with

MS1. These results contrast with previous works which have reported limits of marketability of fresh-cut persimmon 'Rojo Brillante' to fall within the range of 6-8 days by 10 g L^{-1} AA or CA application (Sanchís et al., 2015). The color evaluation of the samples also showed the effectiveness of antioxidant dips to maintain enzymatic browning with high L* and low a* values during storage for both MSs (Figure 1). These differences between color evaluation and visual quality of the persimmon processed at MS1 were due to the development of the physiological disorder known as 'internal flesh browning', which has been identified as being different from enzymatic browning. The cause of this disorder, which appears during the commercialization period, remains unknown. Recent studies have correlated the incidence of this disorder in 'Rojo Brillante' persimmon with the level of insoluble tannins and mechanical damage during packing operations, which suggests a tannin oxidation process (Novillo et al., 2014). Figure 4 shows the appearance of persimmon slices on storage day 7, where the difference between enzymatic browning in the control samples and 'flesh browning' in the antioxidant-treated samples is clearly seen, and which became more evident in the persimmon slices processed with MS1.

The application of antioxidants did not affect the overall flavor of the fresh-cut 'Rojo Brillante' persimmons, which remained within the range of acceptability throughout storage at 5 ºC and did not give rise to any off-flavor in the samples (Table 1). The firmness evaluation confirmed the results obtained in the texture analysis. Upon processing, the persimmon samples were evaluated as very firm at both MSs. After the 9 days at 5 ºC, the persimmons processed with MS1 were evaluated as slightly firmer than those processed with MS2. Nevertheless, the persimmon slices maintained acceptable firmness after storage for both MSs, probably due to the good firmness of the samples at harvest. The selection of an optimum maturity stage was necessary to reduce firmness loss of fresh-cut fruits. Other studies have shown loss of membrane integrity and significant reduced firmness when partially ripe fruits were used in minimal processing (Rojas-Grau et al., 2007; Soliva-Fortuny et al., 2002; Soliva-Fortuny et al., 2004).

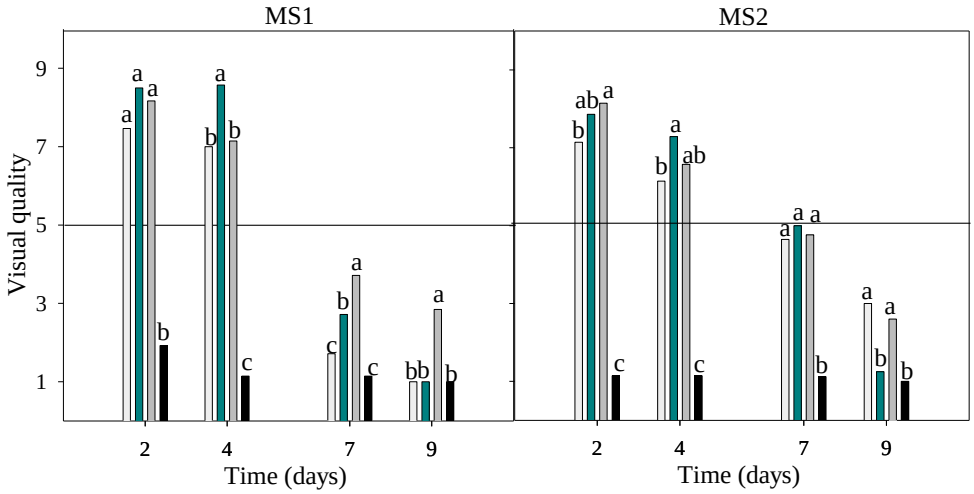

Figure 3. Visual quality of fresh-cut 'Rojo brillante' persimmons dipped in 10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$ (▭), 5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (▬), 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (▬), and water as control (▬) for 9 days at 5 ºC. Persimmons were processed at two maturity stages (MS1 and MS2). The results are the average values. Bars with a different letter differ significantly at the 95% level (n=12).

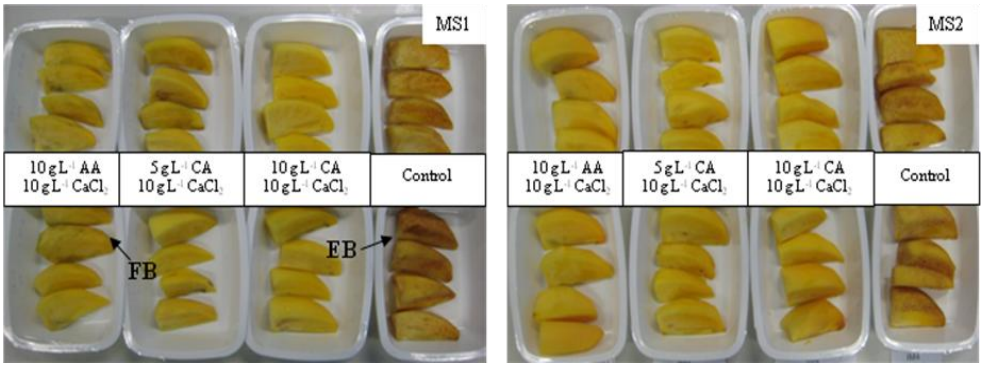

Figure 4. Persimmon slices processed at two maturity stages (MS) and dipped in different antioxidant solutions or in water as control after 7 days of storage at 5 ºC. Arrows show the differences between 'enzymatic browning' (EB) and 'flesh browning' (FB).

Table 1. Effect of maturity stage (MS) and antioxidant treatment on flavor, off-flavor and firmness of fresh-cut 'Rojo Brillante' persimmons for 9 storage days at 5 ºC.

		MS1			MS2		
Day	Treatment	Flavor	Off-flavor	Firmness	Flavor	Off-flavor	Firmness
0		7.00 ± 1.18	1.00 ± 0.00	4.29 ± 0.47	8.00 ± 0.78	1.00 ± 0.00	4.00 ± 0.78
2	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	6.87 ± 1.19a	1.20 ± 0.56a	4.07 ± 0.46a	7.00 ± 1.25a	1.27 ± 0.59a	3.40 ± 0.74a
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.63 ± 1.22a	1.13 ± 0.26a	4.13 ± 0.46a	6.87 ± 1.22a	1.07 ± 0.77a	3.60 ± 0.63a
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.37 ± 1.76a	1.26 ± 0.35a	3.89 ± 0.70a	6.19 ± 1.10a	1.19 ± 0.72a	2.88 ± 0.52a
	Control	6.27 ± 1.45a	1.33 ± 0.56a	3.53 ± 0.64a	6.82 ± 1.03a	1.18 ± 0.52a	2.94 ± 0.68a
4	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	7.07 ± 1.93a	1.07 ± 0.50b	4.07 ± 0.50a	6.93 ± 1.51a	1.20 ± 0.26a	3.40 ± 0.74a
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	7.00 ± 1.03a	1.13 ± 0.34b	3.88 ± 0.62a	6.73 ± 1.49a	1.07 ± 0.26a	2.93 ± 0.70b
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.21 ± 1.32a	1.47 ± 0.81a	3.84 ± 0.89a	6.56 ± 1.96a	1.44 ± 0.41a	2.69 ± 0.70b
	Control	6.40 ± 1.36a	1.20 ± 0.60ab	3.73 ± 0.62a	6.76 ± 1.91a	1.18 ± 1.06a	2.88 ± 0.80b
7	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	6.33 ± 1.67a	1.13 ± 0.56a	4.07 ± 0.91a	6.73 ± 1.22a	1.33 ± 0.40a	3.47 ± 0.50a
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.00 ± 1.78a	1.56 ± 1.02a	4.00 ± 0.69a	6.47 ± 1.36a	1.20 ± 0.81a	2.93 ± 0.95a
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.16 ± 1.71a	1.42 ± 0.69a	3.95 ± 0.71a	6.56 ± 1.31a	1.44 ± 0.81a	2.94 ± 0.85a
	Control	5.60 ± 1.21a	1.53 ± 0.56a	3.87 ± 0.71a	6.41 ± 1.31a	1.41 ± 0.40a	2.71 ± 0.48b
9	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	6.40 ± 1.39a	1.20 ± 0.82a	4.13 ± 0.92a	6.93 ± 1.13a	1.13 ± 0.39a	3.20 ± 0.90a
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.56 ± 1.45a	1.31 ± 0.41a	4.13 ± 0.80a	6.33 ± 1.20a	1.40 ± 0.53a	2.93 ± 0.70a
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.84 ± 1.30a	1.26 ± 0.74a	3.95 ± 0.92a	6.56 ± 1.70a	1.19 ± 0.80a	3.69 ± 0.77a
	Control	6.47 ± 1.36a	1.20 ± 0.56a	3.53 ± 0.74a	6.59 ± 1.42a	1.29 ± 0.77a	3.18 ± 0.81a

For each maturity stage and storage time, small letters indicate significant differences among treatments.

Bioactive compounds

Maturity is one of the major factors that determine the compositional quality of fruits. However, this effect depends on the commodity. For example, total vitamin C was higher in riper apricots, peaches or papayas, whereas riper apples and mangoes presented lower total vitamin C values (Lee and Kader, 2000). In this work, the total vitamin C content of 'Rojo Brillante' persimmon at harvest was, on average, 141 and 162 mg AA/100 g FW for MS1 and MS2, respectively (Table 2). These concentrations fell within the same range as those obtained in other studies for non astringent persimmon cultivars and astringent cultivar 'Rojo Brillante' during similar harvest periods (Del Bubba et al., 2009; Wright and Kader, 1997a). Processing and storage at 5 ºC did not affect the initial total vitamin C values, except in the samples with MS2 that initially decreased after 2 and 4 processing days, with an overall increase seen after 9 storage days. For other commodities, the effect of cutting and storage at 5 ºC on total vitamin C was variable. Gil et al. (2006) reported an increased total vitamin C during storage at 5 ºC for 9 days for pineapple pieces and strawberry slices, but it lowered in fresh-cut mangoes, cantaloupes, watermelons and kiwi fruits. In fresh-cut 'Fuyu' persimmon, Wright and Kader (1997a) reported a loss in total vitamin C on the first day after cutting, but values then recovered to levels that were not significantly different from the first day. In general terms, the application of antioxidant treatments did not affect the total vitamin C of fresh-cut 'Rojo Brillante' persimmon, except that some storage periods showed significant differences among treatments. However, these differences can be attributed to some biological variation since they displayed an erratic trend which makes reaching a conclusion impossible. In some works, the AA application increased total vitamin C in fresh-cut fruits such as mangoes (Robles-Sánchez et al., 2013) and pineapples (González-Aguilar et al., 2005).

Table 2. Effect of maturity stage (MS) and antioxidant treatment on total vitamin c and radical scavenging activity of fresh-cut 'Rojo Brillante' persimmon.

		Vitamin C (mg AA/100g)		Radical scavenging activity (EC_{50}) (g sample/Kg DPPH)	
Day	Treatment	MS1	MS2	MS1	MS2
0		141.2 ± 26.1A	161.3 ± 38.7B	237.2 ± 13.6 B	290.5 ± 38.8A
2	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	168.7 ± 14.2aA	84.9 ± 24.9bA	234.4 ± 20.6bA	274.1 ± 23.7bA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	157.7 ± 25.4aA	149.4 ± 25.6aA	296.9 ± 23.4aA	381.6 ± 27.8aA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	234.6 ± 44.3aA	92.0 ± 16.0abB	110.7 ± 12.9cB	383.0 ± 41.9aA
	Control	177.0 ± 19.3aA	76.7 ± 10.0bB	330.5 ± 26.1 aA	365.4 ± 31.2abA
4	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	192.5 ± 10.4abA	108.7 ± 12.2bB	266.5 ± 15.5aA	283.5 ± 25.0cA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	232.1 ± 24.2aA	68.1 ± 5.1cB	264.5 ± 15.2aB	355.1 ± 29.2bcA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	184.8 ± 12.0bA	74.2 ± 12.9cB	134.3 ± 11.0cB	537.1 ± 36.6aA
	Control	166.3 ± 8.2bA	140.3 ± 7.8aB	209.2 ± 24.2B	442.5 ± 34.4abA
9	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	151.3 ± 16.3aA	223.2 ± 38.7aA	182.9 ± 17.0bB	306.1 ± 17.1bA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	177.5 ± 21.5aA	200.6 ± 28.6aA	264.8 ± 15.3aB	348.9 ± 12.0abA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	144.4 ± 12.7aA	232.0 ± 36.7aA	296.0 ± 8.9aB	363.2 ± 11.6abA
	Control	171.8 ± 21.4aA	220.5 ± 35.6aA	261.6 ± 11.6aB	389.0 ± 30.9aA

AA=ascorbic acid; CA=Citric acid.
For each maturity stage and storage time, small letters indicate significant differences among treatments.
For each treatment, capital letters indicate significant differences between maturity stages.

Radical scavenging activity was expressed as the persimmon extract required to reduce the $DPPH^{\bullet}$ (EC_{50}) by 50%, thus the lower the value, the greater the antiradical capacity of fresh-cut persimmon (Table 2). The fruits harvested at the beginning of October (MS1) presented greater radical scavenging activity than those harvested in mid-November (MS2). However in a recent work, the antioxidant capacity given by the free radical scavenging activity of fresh-cut 'Rojo Brillante' persimmon was significantly greater in the fruits harvested at the end of the season than those harvested earlier (Sanchís et al., 2015). Del Bubba et al. (2009) investigated changes in radical scavenging activity during the growth and maturation of 'Kaki Tipo' and 'Rojo Brillante' persimmon. In the final maturation stage, between mid-October and mid-November, free radical scavenging activity decreased in 'Kaki Tipo', whereas 'Rojo Brillante' persimmon showed erratic behavior with a saw tooth pattern. In the present work, wide variability in the EC_{50} values was also recorded during storage at 5 ºC and values ranged between 40 and 450 g sample per Kg of $DPPH^{\bullet}$. This variability makes it impossible to conclude about the effect of antioxidant treatments on the radical scavenging activity of minimally processed persimmons. Other works found a greater antioxidant capacity of fresh-cut apples (Cocci et al., 2006), mangoes (Siddiq et al., 2013) or kiwi fruits (Antunes et al., 2010) in antioxidant-treated samples than in control samples.

Table 3. Effect of maturity stage (MS) and antioxidant treatment on phenolic content and retinol equivalents (RE) of fresh-cut 'Rojo Brillante' persimmon.

		Total phenolic content (mg GA/100g)		RE (μg /100g)	
Day	Treatment	MS1	MS2	MS1	MS2
0		10.82 ± 0.42A	8.23 ± 0.64B	19.99 ± 1.42A	24.58 ± 1.17A
2	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	9.89 ± 0.32bA	7.72 ± 0.43aB	15.96 ± 1.51aB	26.02 ± 0.38aA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	8.15 ± 0.37bA	7.16 ± 0.40aA	11.61 ± 1.69aB	27.31 ± 1.00aA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	12.05 ± 0.97aA	6.64 ± 0.44aB	11.37 ± 2.99aA	24.81 ± 2.80aA
	Control	12.52 ± 0.62aA	7.56 ± 0.51aB	9.54 ± 0.48aB	18.33 ± 1.37bA
4	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	9.43 ± 0.81aA	6.94 ± 0.40aB	22.43 ± 0.05aB	26.99 ± 0.41aA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	9.41 ± 0.76aA	6.13 ± 0.43aB	24.06 ± 1.23aA	25.90 ± 1.67aA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	10.95 ± 0.54aA	7.07 ± 0.36aB	17.70 ± 0.33aB	26.01 ± 0.53aA
	Control	10.00 ± 0.33aA	6.12 ± 0.27aB	21.27 ± 3.58aA	19.82 ± 1.82bA
9	10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$	7.13 ± 0.28abA	5.68 ± 0.30aB	21.97 ± 1.05dA	20.20 ± 0.75bA
	5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	7.58 ± 0.37aA	4.94 ± 0.45aB	26.03 ± 0.15cB	30.63 ± 0.27aA
	10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$	6.48 ± 0.45bA	4.99 ± 0.55aA	35.24 ± 0.12aA	12.27 ± 0.35cB
	Control	6.18 ± 0.34bA	5.90 ± 0.53aA	28.78 ± 0.14bA	11.51 ± 0.08cB

AA=ascorbic acid; CA=Citric acid; GA=gallic acid.
For each maturity stage and storage time, small letters indicate significant differences among treatments.
For each treatment, capital letters indicate significant differences between maturity stages.

Total phenolic content displayed the same trend as radical scavenging activity, as phenolic compounds contributed in a large extend to the antioxidant activity of fruits and vegetables (Kaur and Kapoor, 2002). Total phenolic content was significantly greater ($p<0.05$) in the samples processed at MS1 than those processed at MS2 (Table 3). In both MSs, phenolic content lowered after processing and during storage at 5 ºC, with losses of around 35-40% at the end of the storage period. In other fresh-cut fruits, phenolic content varied depending on the fruit and processing conditions. Thus, Gil et al. (2006) reported a moderately reduced phenolic content during storage at 5 ºC in mango and cantaloupe cubes, whereas phenolic content was maintained in fresh-cut kiwi fruit, strawberry or pineapple over 9 storage says. Whereas, Reyes and Cisneros-Zevallos (2003) reported that potato wounding induced the synthesis of phenolic compounds. On the other hand, the total phenolic content of fresh-cut 'Rojo Brillante' persimmon was not significantly affected by the antioxidant treatments. However, AA and CA dips have been reported to maintain higher total phenolic levels in fresh-cut apples (Cocci et al., 2006; Gil et al., 1998) and kiwifruit (Antunes et al., 2010) than untreated fruit, which was attributed to the reducing action of AA that prevented a high phenolic degradation.

In this work, eight carotenoids were identified in 'Rojo Brillante' persimmon: α-carotene, β-carotene and six xantophylles (trans-viloaxanthin, cis-violaxanthin, lutein, zeaxanthin, α-criptoxanthin and β-criptoxanthin). Of these, the major carotenoids were, in order of importance, β-carotene, which represented around 70-75% of total carotenoid content, β-cryptoxanthin, α-cryptoxanthin, and α- carotene (Figure 5). Carotenoid content was not generally affected by processing or storage at 5 ºC. Similar results were reported by Wright and Kader (1997b) in fresh-cut 'Fuyu' persimmons stored in controlled atmospheres. The α-criptoxanthin values remained at around 10 μg/100g FW in both MSs during the storage period. Similar behavior was seen in β-criptoxanthin, with values of around 20-30 μg/100g FW, and with some fluctuations in fruits with MS2. The α-carotene and β-carotene values increased after 9 storage days at 5 ºC in the persimmons with MS1, whereas at MS2, these values were either maintained or lowered at the end of the storage.

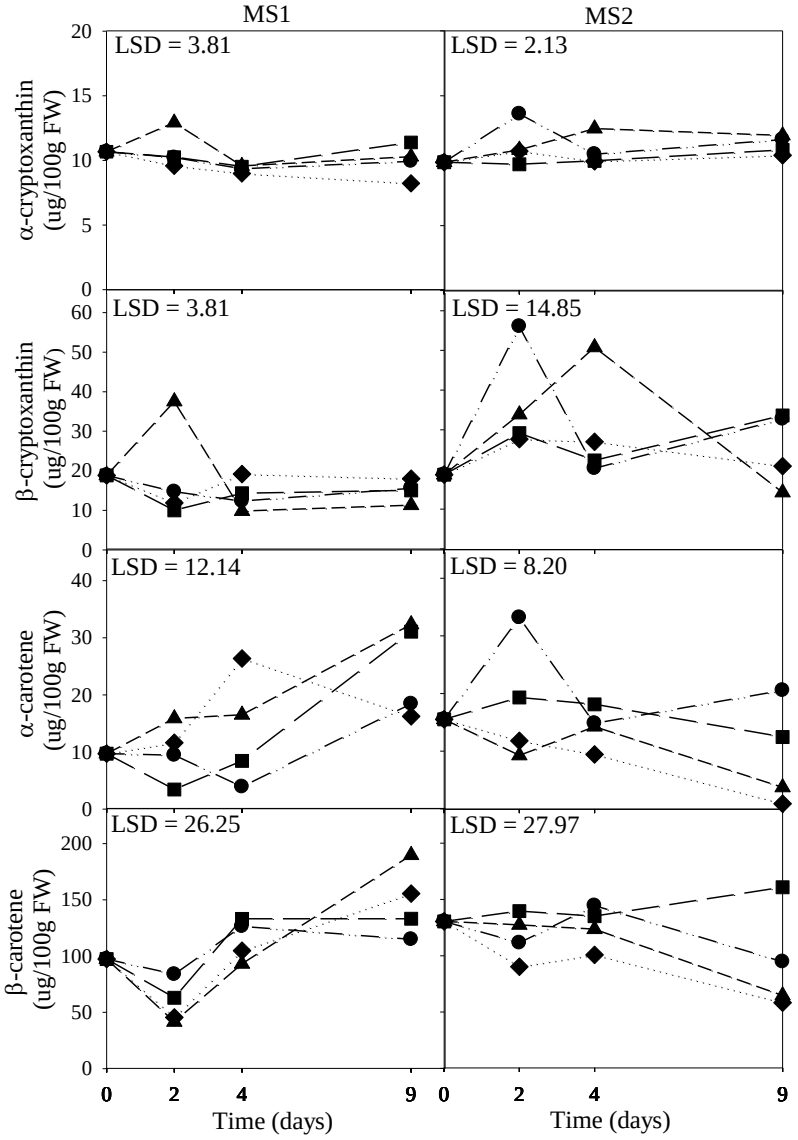

Figure 5. Major carotenoids of fresh-cut 'Rojo Brillante' persimmons dipped in 10 g L^{-1} AA + 10 g L^{-1} $CaCl_2$ (—●—), 5 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (—■—), 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ (– –▲– –), and water as control (◆) for 9 days at 5 ºC. Persimmons were processed at two maturity stages (MS). Pooled LSD at the 5% level (n=3).

Although the effect of antioxidant treatment on carotenoid content of fresh-cut persimmon has not been previously reported, the initial values at harvest were in agreement with the values reported in the literature for β-carotene in 'Rojo Brillante' (Plaza et al., 2012) and for other nonastringent persimmons (Wright and Kader, 1997b; Zhou et al., 2012). However, the relative importance of this carotenoid differed from those reported for 'Rojo Brillante' in other works, where β- cryptoxanthin was the major carotenoid (De Ancos et al., 2000; Plaza et al., 2012; Sanchís et al., 2015). This variability in the contribution of different carotenoids can be attributed to the maturity stage or to the different growing conditions of fruits. Thus for example, lycopene has also been found in 'Rojo Brillante' persimmon harvested at a more advanced maturity stage (De Ancos et al., 2000; Plaza et al., 2012).

By considering the importance of β-cryptoxanthin and of α and β carotene as provitamin A, the retinol equivalent (RE) was also calculated at the two MS studied in this work (Table 3). The RE of 'Rojo Brillante' persimmon ranged between 20 and 24 μg/100 g of FW. These values are of the same order of magnitude as 15 other persimmon cultivars reviewed by Giordani et al. (2011). In 'Rojo Brillante', Plaza et al. (2012) reported RE values of around 22 μg/100 g of FW in fruits in a similar maturity stage. However, de Ancos et al. (2000) obtained values of around 77 μg/100 g of FW for fruits harvested in a more advance ripening stage.

CONCLUSION

Selecting fruits with good firmness and the addition of 10 g L^{-1} $CaCl_2$ help prevent loss of the firmness of fresh-cut 'Rojo Brillante' persimmons treated with acidic solutions as antioxidants to control enzymatic browning. Although all the antioxidant treatments tested proved effective to control enzymatic browning in fresh-cut persimmon, the limit of marketability was conditioned by a burst of the disorder known as 'flesh browning', which negatively affected the visual quality of the samples. Nutritional quality was not negatively affected by cutting, dipping in antioxidant treatments or storage at 5 ºC. Radical scavenging activity and total phenolic content were lower in the fruits harvested at MS2 than those harvested at MS1, and also at the end of the 9-days storage at 5 ºC. Generally speaking, the combination 10 g L^{-1} CA + 10 g

L^{-1} $CaCl_2$ can be used as an antioxidant treatment to commercialize persimmon 'Rojo Brillante' as a fresh-cut commodity for up to 7-8 days at 5 ºC during which time no 'flesh browning disorder' is detected.

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Chapter 3

Effect of postharvest 1-MCP treatment on shelf life of fresh-cut persimmons cv. Rojo Brillante dipped in antioxidants.

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Abstract

Persimmon fruit 'Rojo Brillante' has expanded greatly due to the application of technologies to remove astringency that improves commercialization and transport, maintaining a firm consistency. This allows its commercialization as a fresh-cut commodity; however, the shelf life of minimally processed persimmon is limited due to enzymatic browning and softening. The use of 1-MCP in postharvest is becoming a common technology to prolong the storage time and extend the campaign of persimmons. Thus, the aim of this work was to study the effect of processing on 1-MCP treated persimmon fruit stored at 1 ºC. Persimmon fruit were treated with 600 ppm of 1-MCP and stored at 1 ºC up to 60 days. Untreated fruit was also stored as control. Every 15 days, the astringency of the fruit was removed and cut in slices. Samples treated and untreated with 1-MCP were dipped in an antioxidant solution of 1% citric acid + 1% $CaCl_2$ or water, as control. Persimmon slices were packed under normal atmosphere and stored at 5 ºC for 9 days. Color, firmness and visual quality were measured during storage. Initial L* values decreased with storage at 1 ºC. L* and a* values of fresh-cut persimmons decreased and increased, respectively, during storage at 5 ºC, indicating surface browning. Antioxidant application significantly reduced browning; whereas, 1-MCP had no effect on color of fresh-cut tissue. Fruit firmness decreased during storage at 1 ºC, but 1-MCP reduced firmness loss significantly. The application of 1-MCP at harvest allowed to process persimmon cv. Rojo Brillante after 45 days of storage at 1 ºC. The persimmon slices dipped in the antioxidant solution were evaluated above the limit of marketability after 9 days of storage at 5 ºC.

Keywords: antioxidants, controlled atmosphere, 1-methylcyclopropene, enzymatic browning, firmness, minimally processed persimmon.

INTRODUCION

'Rojo Brillante' persimmon is an astringent cultivar that in the last decade has undergone an important growth in the zone 'Ribera del Xuquer' (Valencia, Spain) due to the application of high levels of CO_2 to remove the astringency while preserving firmness. This technology has also opened the possibility to commercialize the product as fresh-cut commodity. However, the main problem that limits its shelf life is enzymatic browning and softening. The most common way to control enzymatic browning is the use of antioxidants, although their effectiveness for each product depends on their mode of action and concentration. In previous works, the application of 1% ascorbic acid controlled tissue browning of fresh-cut persimmon and maintained the general visual quality above the limit of marketability up to 8 days of storage at 5 ºC (Sanchís et al., 2012) and the combination of 1% citric acid and low O_2 atmospheres extended the storage at 5 ºC up to 9 days (Sanchís et al., 2011). However, softening is still a problem, even when calcium chloride is combined with citric or ascorbic acid (Sanchís et al., 2012).

Persimmon 'Rojo Brillante' presents a short maturation period. Storage at 15 ºC can extend the limit of storability in the packing houses to periods no longer than 20 days due to progressive softening, whereas lower temperatures induce chilling injury (Salvador et al., 2004). 1-Methylcyclopropene (1-MCP), which is an inhibitor of ethylene action, has been effective in alleviating chilling injury symptoms and maintaining firmness of many fruits, including persimmon 'Rojo Brillante' (Salvador et al., 2004). Nowadays, the use of 1-MCP is becoming a common technology in the packing houses to prolong the storage time and extend the campaign of persimmon 'Rojo Brillante'.

Several works have also shown the beneficial effect of 1-MCP on fresh-cut fruits such as kiwifruit, mango and persimmon (Vilas-Boas and Kader, 2007), melon (Ergun et al., 2007), and pears (Lu et al., 2009). Therefore, the objective of this work was to study the effects of postharvest application of 1-MCP, storage time at 1 ºC before processing, and postcutting antioxidant treatment on shelf life of fresh-cut persimmons cv. Rojo Brillante.

MATERIAL AND METHODS

'Rojo Brillante' persimmons were provided by the D.O. 'Kaki Ribera del Xuquer' (Valencia, Spain). Fruit were sealed in 442-L chambers and exposed to either air or 600 μLL^{-1} of 1-methylcyclopropene (1-MCP, *SmartFresh*®, Agro Fresh Inc., Rohm and Hass Co., Philadelphia, PA, USA) for 24 hours at 5 ºC. After 1-MCP or air treatments, persimmons were stored at 1 ºC and 90% RH. After 0, 15, 30, 45, and 60 days of storage, the astringency of the persimmons was eliminated by maintaining the fruit at 20 ºC in closed containers with 95% CO_2 levels for 24 hours. After removal from the containers, fruit were conditioned to 5 ºC for 1 day. Non-astringent persimmons were cleaned, peeled, cut into eight wedges, and dipped in 1% citric acid + 1% $CaCl_2$ (Ant) or in water as control for 3 min. Once dried, 4 pieces were placed in polypropylene trays and sealed with perforated polypropylene films as a secondary package (64 µm thickness) (P12-2050PXNP, Ilpra Systems, Mataró, Spain). To ensure no modification of the surrounding atmosphere, the film was additionally perforated with a needle. Samples were stored up to 9 days at 5 ºC. A sharp stainless-steel knife was used in the process to reduce mechanical bruising and samples were processed in a temperature controlled room at 5 ± 1 ºC. A total of 3 trays were prepared per treatment and storage time at 5 ºC.

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, N.Y., U.S.A.) using the CIELAB color parameters, L*, a*, and b*. Each measurement was taken at 3 locations in a total of 12 sample pieces.

Persimmon firmness was determined using an Instron Universal Machine (model 3343) by measuring the force required for an 8 mm probe to penetrate the sample to a depth of 5 mm at a speed of 5 mm/s. Measurement was made in 12 sample pieces per treatment.

The visual quality for each treatment based on general visual appearance was also determined based on 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). For that, each treatment was coded and presented in random order. A color

photograph of samples rated with this scale was used by judges in the evaluation.

RESULTS AND DISCUSSION

Color Change on Fresh-Cut Persimmon

Surface browning of cut persimmons was accompanied by an increase in a* and a decrease in L* values during storage at 5 ºC. Fig. 1 shows the effects of 1-MCP, storage time at 1 ºC before processing, and antioxidant application on a* values of fresh-cut persimmons 'Rojo Brillante' during storage at 5 ºC.

The application of the antioxidant solution (1% citric acid + 1% $CaCl_2$) to 1-MCP or air-treated samples reduced enzymatic browning compared to control samples (i.e. lower a* values than control); whereas, the 1-MCP treatment and the time of storage at 1 ºC before processing had no significant effect on a* values.

L* values followed a similar trend than a* values. However, differences between antioxidant-treated and non-treated samples were smaller than in a* values (data not shown). These differences decreased as time of storage at 1 ºC before processing increased. Thus, when samples were processed after 45 and 60 days of storage at 1 ºC there were not significant differences among treatments. The application of 1-MCP had no effect on L* values and the time of storage at 1 ºC before processing decreased the lightness of cut tissue (i.e. just after processing) as time increased from 0 to 60 days.

Similar to our results, Vilas-Boas and Kader (2007) also found that different exposure times and concentrations of 1-MCP applied to minimally processed persimmons, mangoes or kiwifruits did not affect the color L* values in comparison with control slices, even when fruits were combined with $CaCl_2$ dip. Similarly, no beneficial effect of 1-MCP treatment reducing enzymatic browning of fresh-cut 'Anjou' pear have been described (Lu et al., 2009); whereas, other work showed a decrease in a* values of 'Blanquilla' pears (Arias et al., 2009).

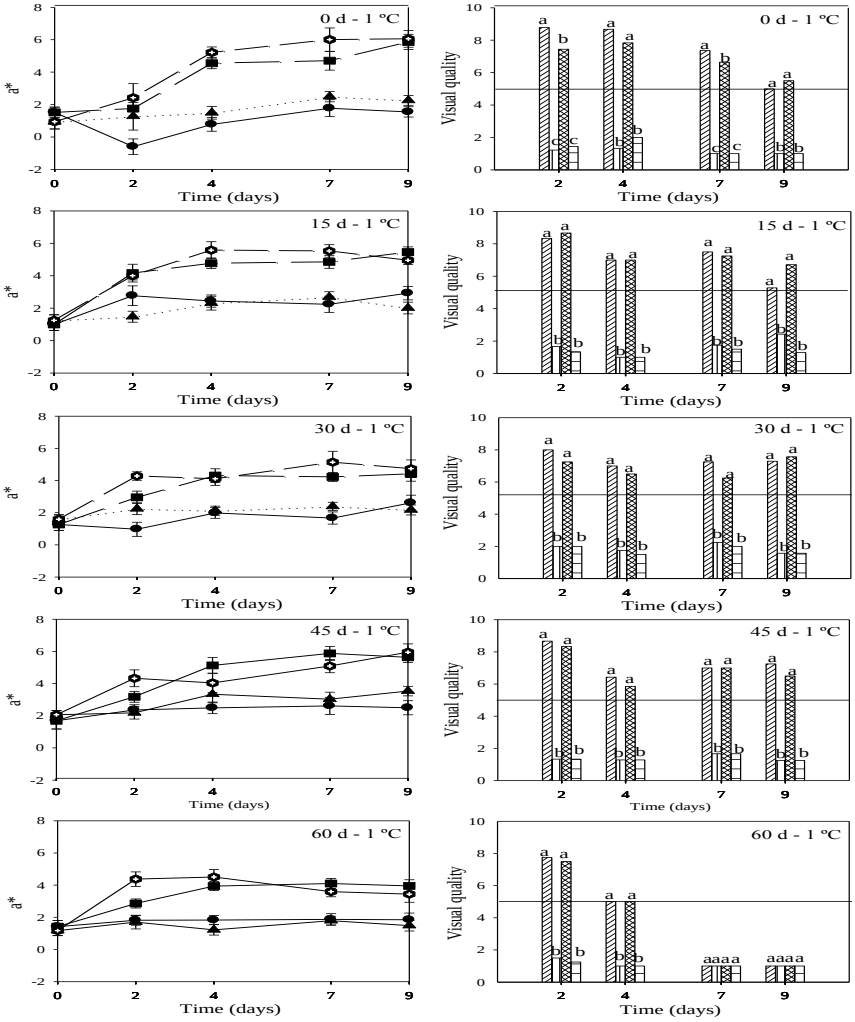

Fig. 1. a* values and visual quality of fresh-cut persimmon 'Rojo Brillante' during storage at 5 ºC: Effect of 1-MCP application, time of storage at 1ºC before processing, and postcutting antioxidant treatment. Cut samples were dipped in 1% citric acid + 1% $CaCl_2$ (w/v) (Ant) or water as control. MCP-Ant ( ), MCP-Control ( ), Air-Ant ( ) and Air-Control ( ). a* values are mean ± standard error. For visual quality, bars with different letter are significantly different at 95% level.

Persimmon Firmness

Fruit firmness of fresh-cut persimmon 'Rojo Brillante' is shown in Table 1. The firmness at harvest of persimmon 'Rojo Brillante' was in the range of 65-73 N and significantly decreased during storage at 1 ºC to values close to 20 N after 60 days. Postharvest application of 1-MCP helped maintaining the firmness in fruit stored 30 and 45 days at 1 ºC, showing higher firmness than untreated samples. However, after 60 days of storage at 1 ºC no differences were observed.

Firmness of fresh-cut persimmon decreased as storage at 5 ºC increased. Although with some fluctuations due to biological variation at the end of the 9 days of storage at 5 ºC, the samples that were initially treated with 1-MCP had higher firmness than those untreated. Several works have also reported the effect of 1-MCP preventing softening during storage of fresh-cut apples (Calderón-López et al., 2005), pineapple (Buda and Joyce, 2003) and papaya (Ergun et al., 2006).

Although some differences were observed between samples treated with the antioxidant and control samples, its application had not significant effect on fruit firmness. However, Antunes et al. (2010) showed that the application of $CaCl_2$ in 1-MCP-treated fresh-cut kiwis reduced the initial losses of firmness due to the cutting process, showing the importance of $CaCl_2$ application maintaining fruit firmness and revealed the loss of the 1-MCP initial protective effect once the fruit was processed.

Visual quality

Visual appearance of persimmon slices, based on color and general appearance, is shown in Fig. 1. Independently of the application or not of 1-MCP, persimmon pieces dipped in the antioxidant treatment were evaluated above the limit of marketability during the 9 days of storage at 5 ºC, except for those that were processed after 60 days of storage at 1 ºC that reached this limit after 4 days of storage at 5 ºC. These results confirm those obtained in color measurements, that showed no significant effect of 1-MCP treatment and time of storage at 1 ºC before processing on a* values.

Table.1. Firmness of fresh-cut persimmon 'Rojo Brillante' during storage at 5 ºC: Effect of 1-MCP application, time of storage at 1ºC before processing, and postcutting antioxidant treatment. Cut samples were dipped in 1% citric acid + 1% $CaCl_2$ (w/v) (Ant) or water as control. Data shown are mean ± standard error.

		Storage time at 1 ºC before processing				
Storage 5 ºC	Treatment	0 days	15 days	30 days	45 days	60 days
0	MCP-Ant	72.60 ± 1.76 aA	53.34 ± 1.53 aB	41.69 ± 1.57 aC	36.21 ± 0.85 aC	22.05 ± 1.50 aD
	MCP-Control	72.60 ± 1.76 aA	53.34 ± 1.53 aB	41.69 ± 1.57 aC	36.21 ± 0.85 aC	22.05 ± 1.50 aD
	Air-Ant	64.60 ± 1.58 aA	41.04 ± 2.32 aB	30.71 ± 0.85 bC	26.12 ± 1.22 bCD	18.90 ± 0.29 aD
	Air-Control	64.60 ± 1.58 aA	41.04 ± 2.32 aB	30.71 ± 0.85 bC	26.12 ± 1.22 bCD	18.90 ± 0.29 aD
2	MCP-Ant	53.34 ± 5.14 aA	33.77 ± 2.76 aB	34.86 ± 3.91 aB	22.66 ± 1.89 aC	25.80 ± 2.23 abBC
	MCP-Control	60.03 ± 3.43 aA	26.61 ± 3.28 aB	32.69 ± 3.84 aB	28.67 ± 1.93 aB	28.02 ± 2.50 aB
	Air-Ant	56.54 ± 3.48 aA	33.01 ± 3.99 aB	30.58 ± 3.43 aBC	24.88 ± 2.64 aBC	21.94 ± 1.92 bC
	Air-Control	55.97 ± 2.41 aA	25.90 ± 3.46 aB	27.51 ± 3.13 aB	24.62 ± 2.31 aB	23.27 ± 1.48 abB
5	MCP-Ant	61.71 ± 2.77 aA	35.68 ± 2.81 aB	19.47 ± 1.59 bC	25.36 ± 2.56 abC	24.70 ± 1.03 aC
	MCP-Control	64.44 ± 3.29 aA	41.23 ± 3.52 aB	30.14 ± 3.31 aC	28.26 ± 1.70 aC	26.47 ± 1.18 aC
	Air-Ant	43.74 ± 2.56 bA	44.49 ± 3.54 aA	15.07 ± 2.68 bB	21.72 ± 2.48 bB	17.34 ± 2.71 bB
	Air-Control	55.28 ± 3.96 aA	40.39 ± 3.64 aB	17.54 ± 2.53 bC	24.97 ± 1.61 abC	22.92 ± 1.85 aC
7	MCP-Ant	49.96 ± 2.82 abA	23.53 ± 2.43 bB	19.93 ± 1.48 aB	24.05 ± 2.80 aB	23.65 ± 1.76 bB
	MCP-Control	57.04 ± 3.14 aA	21.92 ± 1.84 bC	23.18 ± 2.29 aC	24.48 ± 1.81 aC	33.32 ± 2.53 aB
	Air-Ant	42.78 ± 2.66 bA	31.74 ± 3.38 aB	10.20 ± 1.69 bD	20.90 ± 2.80 abC	19.62 ± 1.89 bC
	Air-Control	43.40 ± 3.01 bA	34.99 ± 2.82 aB	12.04 ± 0.92 bD	17.35 ± 1.64 bCD	20.06 ± 1.52 bC
9	MCP-Ant	50.63 ± 3.46 bA	20.35 ± 2.60 bCD	14.93 ± 1.51 aD	31.56 ± 3.00 aB	27.97 ± 2.58 aBC
	MCP-Control	61.23 ± 2.89 aA	30.56 ± 2.62 aB	17.35 ± 1.94 aC	27.43 ± 3.03 aB	29.71 ± 2.44 aB
	Air-Ant	51.65 ± 3.39 bA	20.03 ± 1.90 bB	9.69 ± 1.54 bC	18.24 ± 2.56 bB	19.66 ± 1.74 bB
	Air-Control	49.21 ± 2.78 bA	30.38 ± 3.00 aB	8.13 ± 1.45 bD	19.01 ± 2.39 bC	21.27 ± 1.76 bC

In each column, small letters indicate significant differences among treatments for each storage time at 5 ºC. In a raw, capital letters indicate significant differences among storage periods at 1 ºC. ($p \leq 0.05$).

CONCLUSION

1-MCP application reduced softening of fresh persimmon significantly during storage at 1 ºC. However, its application had no effect reducing enzymatic browning of fresh-cut persimmon 'Rojo Brillante'. Samples treated with 1% citric acid + 1 % $CaCl_2$ prevented enzymatic browning of cut persimmon and helped maintaining a good visual quality during 9 days at 5 ºC in samples processed up to 45 days of storage at 1 ºC. The combination of 1-MCP treatment at harvest and the postcutting antioxidant treatment can prevent both enzymatic browning and softening, extending the campaign of persimmon 'Rojo Brillante' and allowing to commercialize fresh-cut persimmon with good quality.

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Chapter 4

Effect of controlled atmosphere storage and antioxidant dips on the physico-chemical, visual and nutritional quality of minimally processed 'Rojo Brillante' persimmons

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Abstract

The combined effect of antioxidant dips and controlled atmosphere storage on fresh-cut 'Rojo Brillante' persimmon quality was investigated. Persimmon slices were dipped in 1% (w/v) ascorbic acid (AA), 1% (w/v) citric acid (CA) or water, and were stored in different controlled atmospheres at 5 ºC. The study was divided into two experiments: in experiment 1, the control atmosphere conditions were 21 kPa O_2 + 10 kPa CO_2 (Atm-B), 21 kPa O_2 + 20 kPa CO_2 (Atm-C) and 5 kPa O_2 + 10 kPa CO_2 (Atm-D); in experiment 2, persimmon slices were stored in 5 KPa O_2 (Atm-E) and compared to Atm-D. In both experiments, air (Atm-A) was used as a control. The studies were conducted in fruits harvested in two maturity stages (MS). Atmospheres with high CO_2 concentrations induced darkening in some tissue areas, associated with a flesh disorder known as 'internal flesh browning'. Only the samples placed in Atm-E, and treated with 1% AA or 1% CA, controlled enzymatic browning, reduced firmness loss and prevented the 'internal flesh browning' disorder. The maximum limit of marketability was achieved in the samples treated with 1% CA and stored in Atm-E for 9 storage days at 5 ºC, irrespectively of MS. The total vitamin C, free radical scavenging activity, total phenolic content, and total carotenoids of the fresh-cut 'Rojo Brillante' persimmons were not influenced by controlled atmosphere storage. However, the application of antioxidants prevented some nutritional loss.

Keywords: Fresh-cut; Firmness; Ascorbic acid; Citric acid; Browning; Bioactive compounds.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) production is widely extended worldwide and has presented an upward trend in the last decade, mainly in the Mediterranean region with the expansion of cultivar 'Rojo Brillante' (Valencia, Spain). This cultivar is very much appreciated in European markets for its size, color and flavor, and because it is a good source of bioactive compounds (Plaza et al., 2012). This fruit is astringent at harvest, but the application of high CO_2 concentrations allows astringency to be removed, while fruit firmness is preserved. This technology also enables the commercialization of 'Rojo Brillante' persimmon fruit as a fresh-cut

commodity. However, minimally processing leads to enzymatic browning and softening, which significantly reduces the product's shelf life (Sanchís et al., 2015). Several physical and chemical treatments, such as antioxidant dips and modified atmosphere storage, may be applied in synergy with proper temperature management to extend the shelf life of fresh-cut fruits. In recent works, dips in antioxidant solutions of 1% ascorbic acid (AA) or 1% citric acid (CA) have controlled the tissue browning of fresh-cut 'Rojo Brillante' persimmons and maintained visual quality above the limit of marketability by up to 6-8 storage days at 5 ºC. The limit of marketability was strongly affected by the fruit's maturity stage at harvest (Ghidelli et al., 2013; Sanchís et al., 2015).

The successful application of modified atmosphere packaging with low O_2 and high CO_2 for fresh-cut fruits and vegetables has been extensively reported in the literature, and optimal atmospheres have been recommended for some fresh-cut fruits and vegetables (Gorny, 2003). However, caution must be taken in applying the recommended atmospheres since a product may respond in various ways as a result of differences in physiological maturity, growing conditions, postharvest handling conditions prior to cutting, and the expected storage/distribution temperature. Therefore, studying the efficacy of a given recommendation for a specific situation before applying it in commercial practice is recommended (Toivonen et al., 2009).

The study of controlled atmospheres is generally the first step to select optimum O_2 and CO_2 concentrations for modified atmosphere packaging. Wright and Kader (1997a) reported that 'Fuyu' persimmons slices stored under controlled atmosphere conditions (2kPa O_2 + 12kPa CO_2) maintained good visual quality for up to 8 storage days at 5 ºC, whereas areas of faint black pigmentation had begun to develop on the fruit stored in air. This cultivar also showed loss in vitamin C and carotenoid content during controlled atmosphere storage after being cut (Wright and Kader, 1997a, 1997b). As far as we know, no works have reported the effect of controlled atmospheres or modified atmosphere packaging on the commercial shelf life and bioactive compounds of fresh-cut 'Rojo Brillante' persimmons. Therefore, the aim of this study was to evaluate the effect of different controlled atmospheres, in combination with ascorbic or citric acid dips on

the physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmons harvested in two commercial maturity stages.

2. Material and methods

This study was conducted during two growing seasons and included two experiments. In the first experiment, the study was done to identify successful combinations of antioxidants and different controlled atmosphere conditions. In the second experiment, selected controlled atmosphere conditions were tested in the persimmon fruits harvested in two different commercial maturity stages (MSs).

Reagents

Persimmon fruits (*Diospyros kaki* cv. Rojo Brillante) were provided by the Protected Designation of Origin (PDO) Kaki Rivera del Xúquer (Valencia, Spain). Ascorbic acid (AA) and citric acid (CA) were supplied by Quimivita (Barcelona, Spain). 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, sodium chloride, ammonium acetate, β-apo-8'-carotenal and β-carotene were obtained from Sigma (St. Louis, MO, USA). Methanol, chlorhydric acid, ethanol, hexane, methylene chloride, acetonitrile and butylated hydroxytoluene were purchased from Scharlau (Barcelona, Spain). β-cryptoxanthin, lutein, lycopene and zeaxanthin were supplied by Extrasynthese (Genay, France). Gallic acid came from Acros Organics (Geel-Belgium) and thriethylamine from Panreac (Barcelona, Spain). All the solvents were of HPLC-grade and Milli-Q system ultra-pure water (Millipore Corp., USA) was used throughout this research work.

Plant material and sample preparation

Persimmon fruits were harvested during two persimmon seasons at commercial MS determined by external color as a color index (CI=1,000 a/L b using the Hunter L, a, b color space) (Salvador et al., 2007). For experiment 1, fruits were harvested in mid-November and had a CI of 8.4±0.3 and flesh firmness of 40.0±0.5 N. For experiment 2, persimmons were harvested in early October (MS1) and mid-November (MS2), which corresponded to the beginning and end of the season, respectively. The CI

and flesh firmness were -0.6±0.2 and 65.6±0.4 N for MS1 and 14.1±0.7 and 41.5±2.5 N for MS2, respectively.

Before processing, astringency was removed according to commercial practices by applying 95% of CO_2 in closed containers for 24 h at 20º C and at 90% relative humidity (RH) (Arnal and del Rio, 2003). The persimmons pre-cooled at 5±1ºC for 20 h were washed with chlorinated water (150 mg L^{-1}), peeled and cut into eight wedges. Pieces were dipped for 3 min in 1% (w/v) AA, 1% (w/v) CA or water as a control, and were allowed to drain and dry at 5±1ºC before storage under controlled atmosphere conditions.

Controlled atmosphere storage treatments

In experiment 1, the controlled atmosphere treatments of air + 10 kPa CO_2 (Atm-B), air + 20 kPa CO_2 (Atm-C), and 5 kPa O_2 + 10 kPa CO_2 (Atm-D) were compared to air (Atm-A). In experiment 2, the controlled atmosphere treatments of 5 kPa O_2 + 10 kPa CO_2 (Atm-D) and 5 KPa O_2 (Atm-E) were compared to air (Atm-A). All the gas mixtures were balanced with N_2. Fruit slices were placed in 2-L glass jars at 5 ºC under a continuous air flow or the specified gas mixture humidified by passing through distilled water to maintain 90-95% RH. The flow rate was 35 mL/min to prevent ethylene accumulation. The gas composition, as supplied to the jars, was measured with a gas analyzer (PBI Dansensor, Check Mate 9900, Ringsted, Denmark). Persimmon slices were evaluated for up to 7 and 9 days for experiment 1 and 2, respectively.

Quality evaluation

The physico characteristics of the persimmon fruits before processing were evaluated in 30 fruits for external color (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Osaka, Japan) and firmness (Instron Universal Machine, Model 3343, Instron Corp., Canton, MA, USA). External color was expressed as the CI using the Hunter L, a, b color space and fruit firmness as the maximum force in newtons (N) required to penetrate fruit flesh after removing skin in the equator using a 8-mm diameter probe.

In fresh-cut persimmons, color and firmness were determined on 12 pieces per treatment and sampling day. The CIE *L*a*b** color space was used to evaluate flesh color. Each measurement was taken randomly at three

locations per sample piece. Fresh-cut persimmon firmness was evaluated as the force (N) required for an 8-mm diameter probe to penetrate the sample to a depth of 2 mm at a speed of 5 mm/s.

The visual quality of persimmon slices was conducted by 15 trained judges. Each treatment was presented on trays that contained 12 persimmon pieces to account for sample variability, labeled with a 3-digit random code and presented to the judges under the same conditions (light intensity and temperature) to minimize variations in human perception. Visual quality, based on general visual appearance, was determined on 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 color photograph of the samples rated with this scale was used to score samples.

Bioactive compounds

Total vitamin C (TVC), free radical scavenging activity, total phenolic content (TPC), and carotenoids were evaluated in fresh-cut 'Rojo Brillante' persimmons processed in two different MSs and stored 2, 5 and 9 days at 5 ºC under the different atmosphere conditions tested in experiment 2. Each sampling day, 18 persimmon slices per treatment were frozen in liquid nitrogen and kept at -80ºC until analyzed. Bioactive compounds were determined in 3 replicates per treatment.

Total vitamin C (TVC) was determined as the sum of ascorbic acid and L-dehydroascorbic acid as described by Wright and Kader (1997a). Two grams of persimmon samples, which had been stored at –80 ºC, were homogenized with 38 mL of a solution of 0.1 M citric acid and 0.05% ethylenediaminetetraacetic acid in 5% aqueous methanol for 2 min at 22000 rpm (Ultraturrax, IKA, Germany). Two mg of D-isoascorbic acid were added as an internal standard. The homogenate was centrifuged at 10000 rpm for 5 min at 4 ºC. Next 1.5 mL of supernatant was reacted with 0.5 mL of 1,2-phenylenediamine (3.33 mg/mL) and diluted in methanol:water (5:95, v/v). The mix was kept for 37 min in the dark at room temperature. Afterward samples were passed though a 0.45 μm membrane filter into an amber vial and sealed to be analyzed by high pressure liquid chromatography (HPLC). The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-

2200), quaternary pump (Model L-2130), a column oven (Model L-2300), and a diode array detector (Model L-2450). A reversed-phase C18 LiChroCart® column (250 x 4 mm, 5 μm particle, Merck, Darmstadt, Germany), preceded by a precolumn (4 x 4 mm), was used. The injection volume was 40 μL and the oven temperature was 4 ºC. The mobile phase was a methanol:water solution (5:95, v/v) that contained 5 mM of hexadecyltrimethylammonium bromide and 50 mM of ammonium dihydrogen phosphate, adjusted to pH 4.6. The flow rate was fixed at 1 mL/min. Ascorbic acid and D-isoascorbic acid were detected at 261 nm, whereas L-dehydroascorbic acid was detected at 348 nm. TVC was expressed as mg of TVC per 100 g sample.

The free radical scavenging activity of persimmon slices was determined by the method of Brand-Williams et al. (1995) using DPPH• as the free radical. Extraction was done as described by Chen et al. (2008), with some modifications. Two grams of persimmon pulp were mixed with 30 mL of 80% methanol. The solution was homogenized at 20000 rpm for 2 min, followed by boiling in a water bath for 20 min to inactivate the PPO enzyme. The homogenate was immersed in an ultrasonic machine at room temperature for 15 min and centrifuged at 10000 rpm for 20 min at 5 ºC. The resultant supernatant was then filtered and used as the persimmon extract. A second pulp extraction was required to complete extraction. The mix of both extracts was used to analyze the antiradical capacity of the samples. Five methanolic dilutions from the supernatant were prepared to relate the decrease in DPPH• absorbance with sample concentration. Seventy five μL of extract were mixed with 225 μL of DPPH• (24 ppm) and the mixture was kept in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 520 nm using a multiplate spectrum (Multiskan Spectrum, Thermo Fisher Scientific, Finland). DPPH• radical scavenging activity was expressed as an effective concentration (EC50). This value expresses the amount of persimmon extract required to lower the initial DPPH• concentration by 50%; thus lower EC50 values mean greater antiradical capacity. Radical scavenging activity was expressed as g of persimmon fruit per kg of DPPH•.

The total phenolic content (TPC) was measured following the method described by Chen et al. (2008). One gram of frozen sample was mixed with 15 mL of methanol with 1% hydrochloric acid. This mix was homogenized

at 10000 rpm for 1 min, immersed in an ultrasonic bath for 30 min and centrifuged at 1000 rpm for 20 min at 4ºC. The supernatant was filtered and collected. Extraction was repeated and the supernatants were combined for the analysis. Two methanolic dilutions were prepared with the extracts. Then 300 µL of supernatant were mixed with 600 µL of Folin Ciocalteu reagent and 2.4 mL of sodium carbonate solution (200 mg/mL), and in this order. The mixture was incubated for 1 h in the dark at room temperature. The absorbance of the resulting solution was measured at 765 nm with a spectrum multiplate reader. The results were expressed as mg of gallic acid per 100 g of persimmon fruit.

Carotenoids were determined as described by Wright and Kader (1997b). For the extraction, 5 g of sample were added to a centrifuge tube together with 10 mL of cold ethanol to be homogenized for 3 min at 16000 rpm. Eight mL of hexane were added and the sample was homogenized for another 2 min. The mixture was then centrifuged for 4 min at 5000 rpm and 4 ºC. The organic phase was transferred to a 250-mL screw-cap Erlenmeyer flask. The extraction was repeated with 5 mL of saturated sodium chloride and 8 mL of hexane. The resultant organic phase was transferred to the Erlenmeyer flask with the first extract. For saponification, 15 mL of 10% methanolic potassium hydroxide were added to the Erlenmeyer flask. The flask was flushed with nitrogen, sealed, covered with aluminum foil to prevent oxygen and light, and left at room temperature for 16 h with gentle shaking. Next the mixture was transferred to a separatory funnel to remove the potassium hydroxide with 15 mL of 10% sodium chloride, followed by deionized water until the pH of the mixture became neutral. The final extract was evaporated under nitrogen until dryness and was kept at -80 ºC until analyzed. At the time of the analysis, samples were redissolved in 200 µL of methylene chloride and 1.8 mL of the mobile phase. The major carotenoids were determined by HPLC. For the analysis, the resuspended sample was filtered into amber vials using a 0.45-µm nylon filter. The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), a quaternary pump (Model L-2130), a column oven (Model L-2300) and a diode array detector (Model L-2450). A reversed-phase C30 YMC-Pack column (250 x 4.6 mm, 5-µm particle size, Merck, Darmstadt, Germany) was used. The injection volume was 60 µL and the oven temperature was 4 ºC. The mobile phase consisted in acetonitrile, methanol containing 0.05 M ammonium acetate and

methylene chloride 75:20:5 (v/v/v) which, in turn, contained 0.1% butylated hydroxytoluene and 0.05% triethylamine. The flow rate was 1.5 mL/min. Detection was done at 450 nm. Identification of peaks was confirmed using the standards of major compounds. The total carotenoid concentration was also quantified in a multiplate spectrum reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The resuspended sample (0.5 ml) was mixed with 2.5 ml of the mobile phase and measured within the 300-500 nm wavelength range. The results were expressed as μg of total carotenoids per g of persimmon.

Statistical analysis

Statistical analyses were performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences among treatments were determined by least significant differences (LSD) when the analysis of variance (ANOVA) showed a significant F-value. Significant differences were defined at $p \leq 0.05$.

3. Results and discussion

Color and firmness

Color *L** and *a** values were selected as the most suitable parameters to measure fresh-cut persimmon surface browning. Fig. 1 shows the color change of the samples for the first experiment, with a decrease in *L** and an increase in *a** as storage at 5 ºC was prolonged. In the control samples (water-dipped), the application of the different controlled atmospheres reduced fresh-cut persimmon enzymatic browning, as observed by the higher *L** and the lower *a** values if compared to those stored in air (Atm-A), where Atm-D (5 kPa O_2 + 10 kPa CO_2) was the most effective. The effectiveness of a similar atmosphere to control browning has been reported in fresh-cut papaya (Waghmare et al., 2013) or mangosteen (Manurakchinakorn et al., 2011), while an atmosphere of 2 kPa O_2 + 12 kPa CO_2 has also been shown to maintain higher *L** and lower *a** than the atmospheric conditions in fresh-cut 'Fuyu' persimmons over 8 storage days at 5 ºC (Wright and Kader, 1997b).

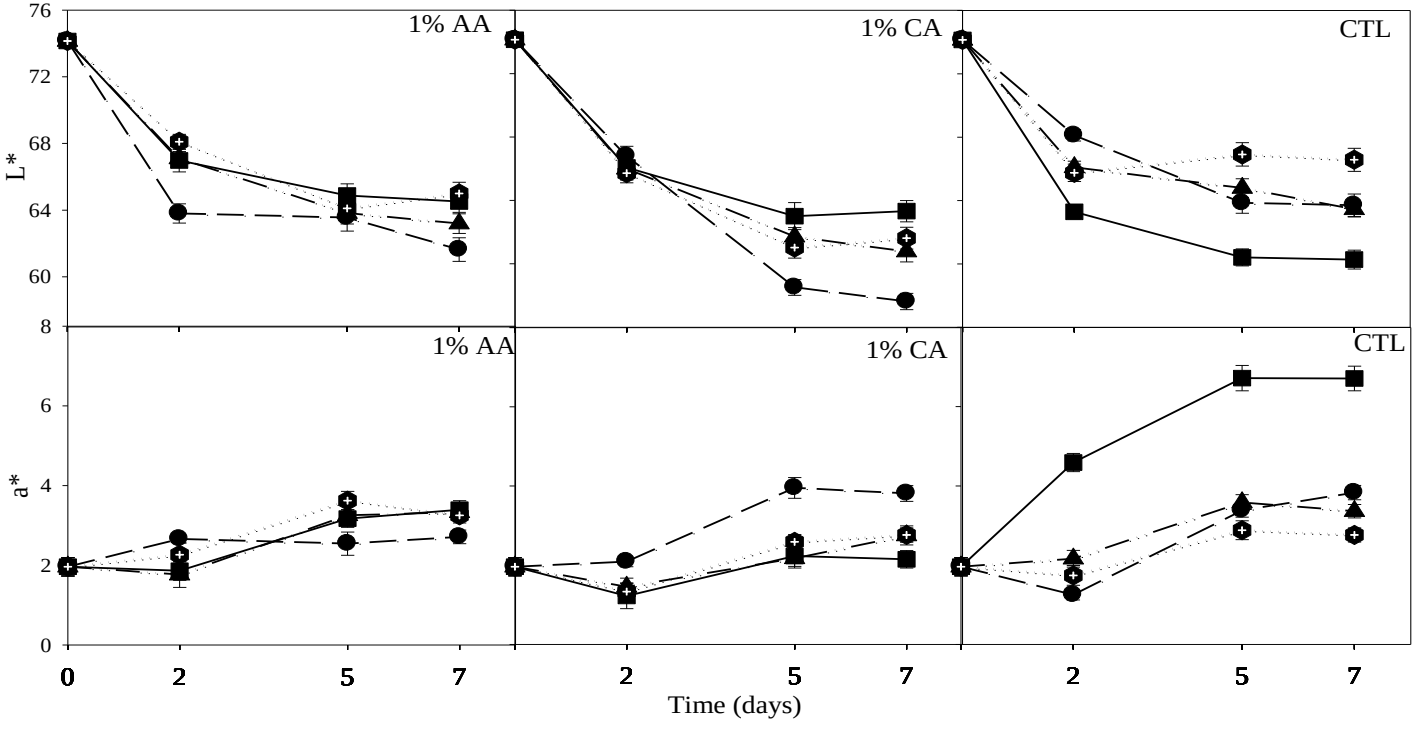

Fig. 1 Color L* and a* changes in fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (—■—), Atm-B (—▲··), Atm-C (—●··) and Atm-D ··⬢··) for 7 days at 5 ºC and dipped in 1% AA (w/v), 1% CA (w/v) or water (CTL). Atm-A = air; Atm-B = air + 10 kPa CO_2; Atm-C = air + 20 kPa CO_2; Atm-D = 5 kPa O_2 + 10 kPa CO_2. Vertical bars are standard errors.

When persimmon slices were dipped in antioxidant solutions, enzymatic browning diminished, whereas the combination of antioxidants and the different controlled atmospheres did not further reduce browning. On the contrary, the application of high CO_2 concentrations induced darkening in some tissue areas, which differed from that observed as enzymatic browning due to the cutting process. Several studies have described this tissue darkening as a flesh disorder in whole persimmons, known as 'flesh browning' (Novillo et al., 2014a, 2014b). Even though the cause of this disorder remains unknown, it has been related to pre-harvest nutritional deficiencies, mechanical injury during the postharvest period and the post-application of high CO_2 atmospheres to eliminate astringency (Besada et al., 2010; Zavrtanik et al., 1999). In recent studies, Novillo et al. (2014a, 2014b) reported that the incidence and severity of 'flesh browning' in 'Rojo Brillante' persimmons was greater the longer the CO_2 exposure time taken to remove astringency. This correlated with an accumulation of superoxide anion and H_2O_2, which suggests the implication of oxidative stress in this postharvest disorder of persimmon fruits. In our work, 'flesh browning' increased as the CO_2 concentration increased, but mainly in those samples dipped in antioxidants, where the persimmon slices that were dipped in 1% CA and placed in Atm-C (air + 20 kPa CO_2) were those that displayed the most 'flesh browning'. Gorny et al. (2002) also observed accelerated tissue browning, as well as necrosis, in fresh-cut pears when they applied similar controlled atmospheres (air + 10% CO_2 and air + 20% CO_2). In their work, substantial CO_2 injury occurred in a dose-responsive manner, and damage occurred earlier and more severely in the 20 kPa CO_2-treated slices than in the 10 kPa CO_2-treated slices.

As the application of atmospheres with high CO_2 concentrations induced 'flesh browning' of fresh-cut 'Rojo Brillante' persimmons, a second experiment was designed in which an atmosphere with 5 kPa O_2 and without CO_2 (Atm-E) was compared with Atm-D (5 kPa O_2 + 10 kPa CO_2) and Atm-A (air conditions) in persimmon fruits harvested at two MS and dipped in 1% AA or 1% CA. The color L* and a* values decreased and increased, respectively, in association with fresh-cut persimmon browning during storage (Fig. 2 and 3). The tested controlled atmospheres only reduced enzymatic browning in the control samples (water-dipped) processed at MS1, whereas the samples harvested late in the season (MS2)

and/or dipped in antioxidants were not systematically affected by atmosphere composition. In the control samples with MS1, Atm-D proved to be the most effective application to prevent enzymatic browning. However for both MSs, the persimmon slices packed in this atmosphere presented some tissue areas with the 'flesh browning' disorder, whereas Atm-E completely prevented this disorder from appearing, which confirms the effect observed in experiment 1. Although very few studies have linked the use of antioxidants with low O_2 atmospheres, some works have shown a synergic effect that reduced browning in fresh-cut fruits. Thus the application of 1% AA, and in association with 0.4 kPa O_2, prolonged the shelf life of carambola slices by up to 12 days at 4.1 ºC (Teixera et al., 2008). Yet the application of antioxidants in the present work significantly decreased enzymatic browning, but overwhelmed the effect of the studied controlled atmospheres, which corroborates the results obtained in experiment 1. The effectiveness of the antioxidants was also less marked in the persimmons harvested late in the season, which confirms previous findings in fresh-cut 'Rojo Brillante' persimmons (Sanchís et al., 2015).

The controlled atmospheres and the antioxidant dips tested in experiment 1 induced tissue softening of persimmon slices if compared to the control samples stored in the air atmosphere (Fig. 4). In experiment 2, persimmon fruits were harvested at higher maturity than in experiment 1. Firmness diminished after processing with an average firmness loss of 14% and 41% for MS1 and MS2, respectively, after 9 days at 5 ºC (Fig. 5). This indicates the importance of firmness at harvest for maintaining sound firmness during storage. The application of antioxidant dips did not affect fruit firmness in the persimmon fruits processed with MS1, but the firmness of the antioxidant dipped-samples was significantly lower in the fruits processed with MS2 (average value of 24±7 N) than in the water-dipped samples (average value of 32±8 N), as observed in experiment 1. Fruit firmness reduced by acid solutions has also been reported in some fresh-cut fruits, such as pears (Oms-Oliu et al., 2006), apples (Rojas-Graü et al., 2007) and persimmons (Sanchís et al., 2015), which indicates some damage to the cell wall structure. The tested controlled atmosphere did not prevent the fruit softening of persimmon slices. Similar results have been reported for fresh-cut pears (Gorny et al., 2002), bananas (Vilas-Boas et al., 2006) or apples (Rojas-Graü et al., 2007) packed in low O_2 and high CO_2 (10-20 kPa) atmospheres.

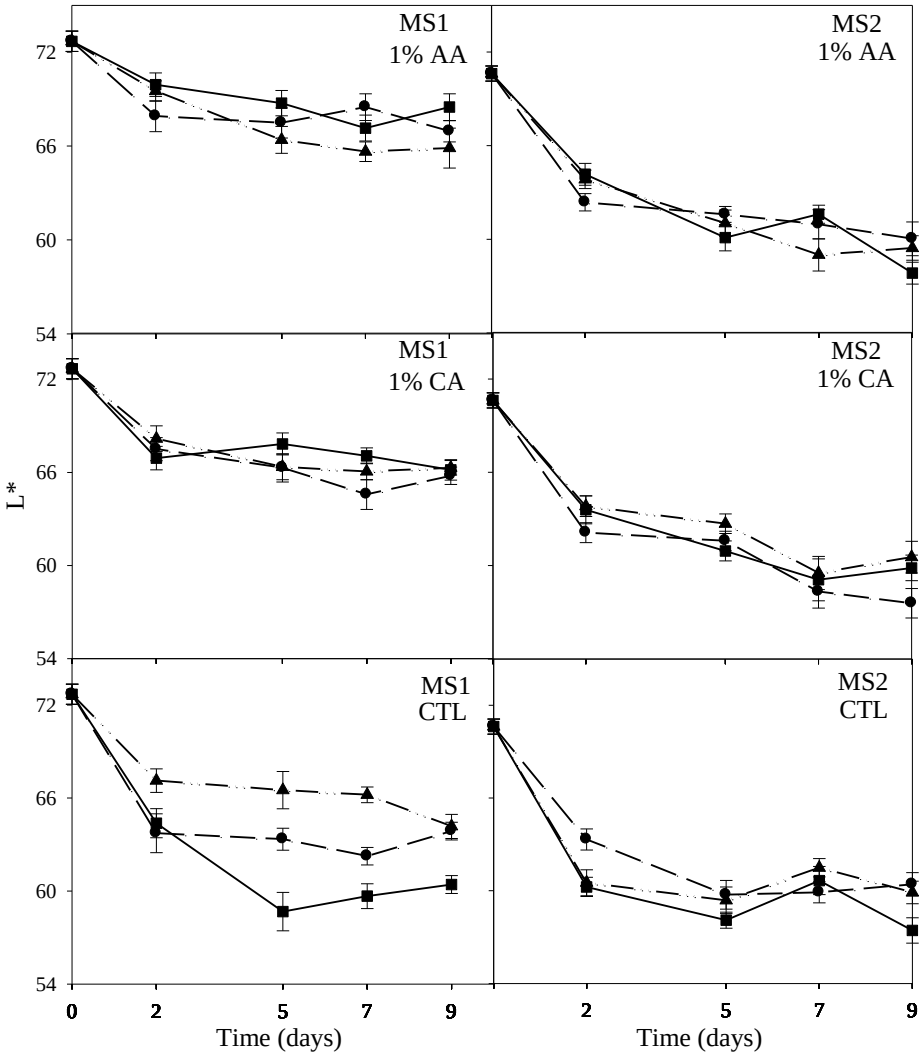

Fig. 2 Color L* changes in fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (—■— Atm-D (—▲ ··) or Atm-E (—● ·) for 9 days at 5 ºC and dipped in 1% AA (w/v), 1% CA (w/v) or water (CTL). Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2. Vertical bars are standard errors.

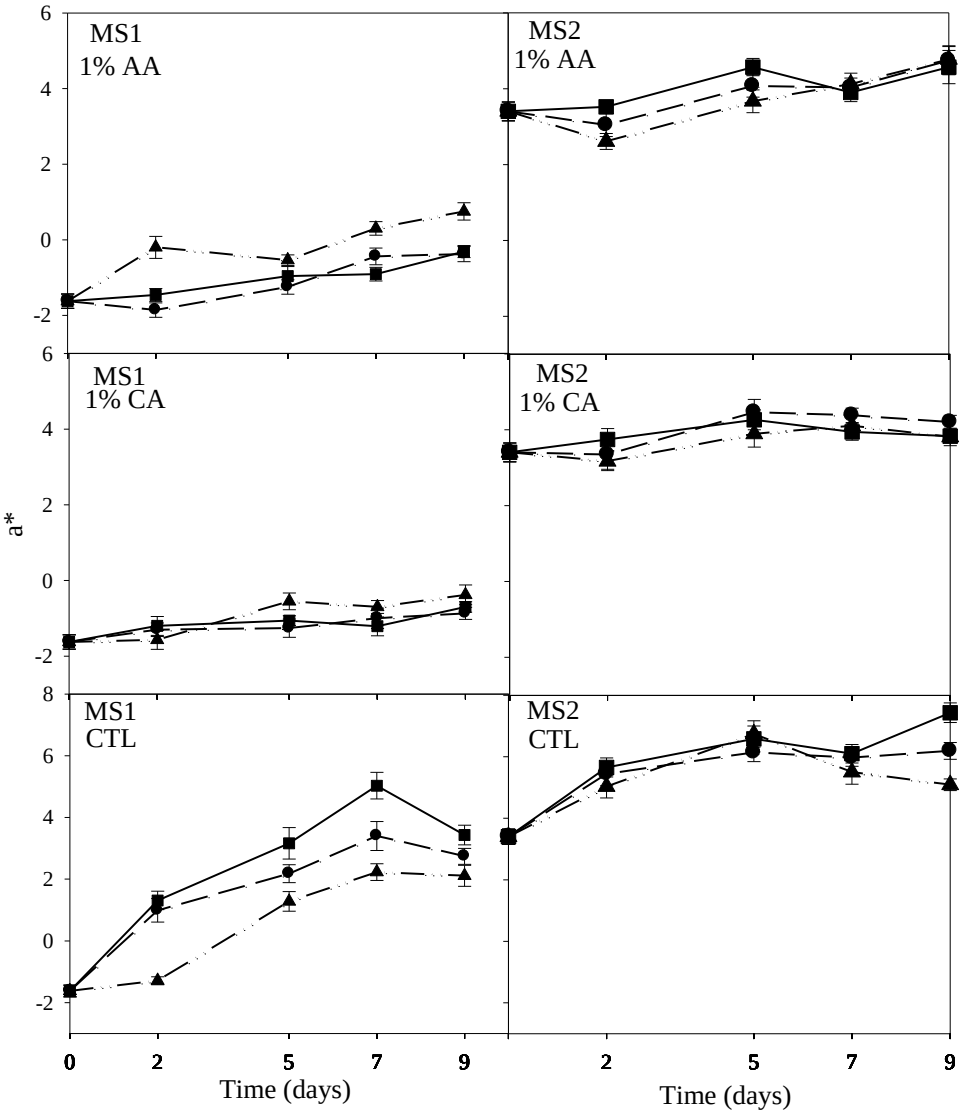

Fig. 3 Color a* changes in fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (—■— Atm-D (—▲··· or Atm-E (—●···) for 9 days at 5 ºC and dipped in 1% AA (w/v), 1% CA (w/v) or water (CTL). Persimmons were processed in two maturity stages (MS1 and MS2). Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2. Vertical bars are standard errors.

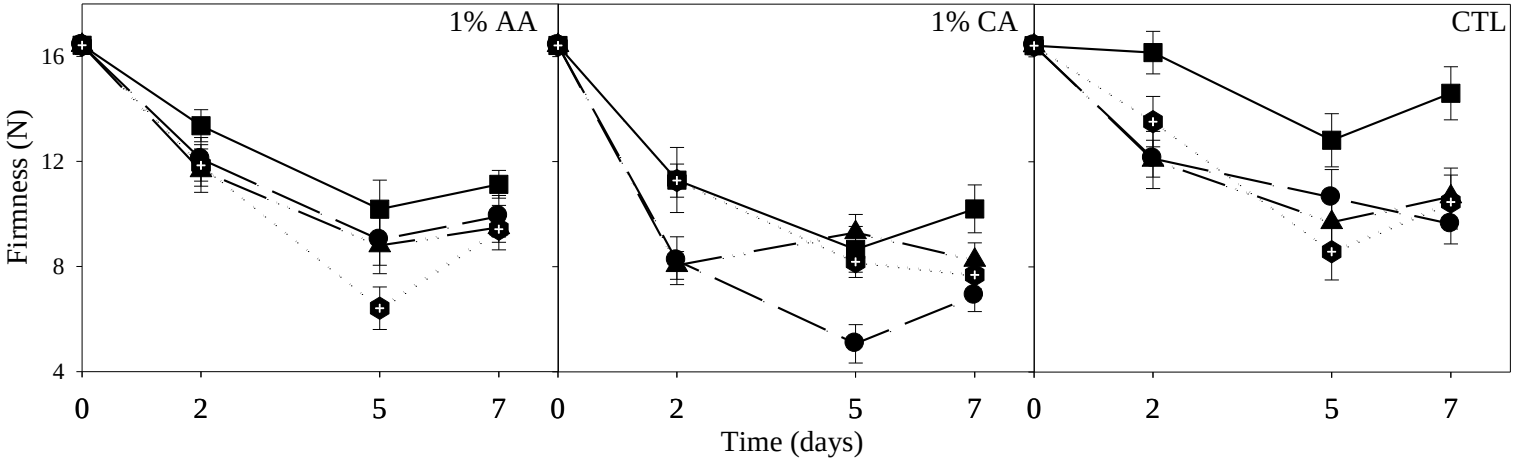

Fig. 4 Firmness of fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (—■—), Atm-B (—▲···), Atm-C (—●···) and Atm-D (···⬢···) for 7 days at 5 ºC and dipped in 1% AA (w/v), 1% CA (w/v) or water (CTL). Atm-A = air; Atm-B = air + 10 kPa CO_2; Atm-C = air + 20 kPa CO_2; Atm-D = 5 kPa O_2 + 10 kPa CO_2. Vertical bars are standard errors.

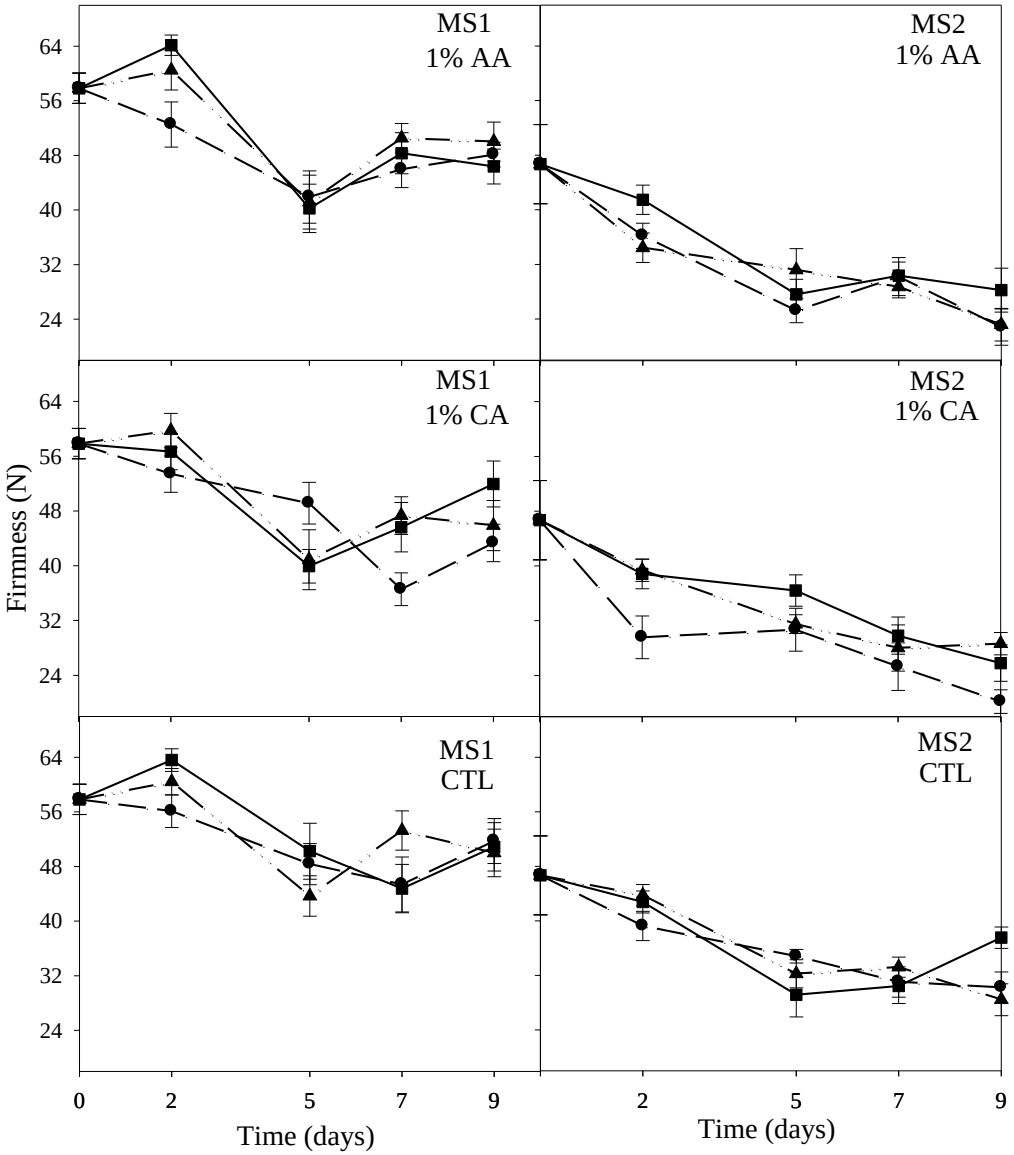

Fig. 5 Firmness of fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (—■—), Atm-D (—▲ ··) or Atm-E (—● ·) for 9 days at 5 ºC and dipped in 1% AA (w/v), 1% CA (w/v) or water (CTL). Persimmons were processed in two maturity stages (MS1 and MS2). Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2. Vertical bars are standard errors.

Visual quality

Fig. 6 presents the visual quality of the fresh-cut persimmons processed in the two MSs and stored for 9 days at 5 ºC in the atmospheres tested in experiment 2. The control samples (water-dipped) were evaluated as poor or inedible by storage day 2, independently of the atmosphere tested and the MS at harvest. This shows that low O_2, either combined or not with high CO_2 conditions, does not suffice to control enzymatic browning in fresh-cut 'Rojo Brillante' persimmons. When 1% AA was applied, the samples stored in Atm-E (5 kPa O_2) and Atm-A (air) reached the limit of marketability by days 7 and 9 for MS1 and MS2, respectively. However, the samples placed in Atm-D (5 kPa O_2 + 10 kPa CO_2) were either below that limit by day 5 for the MS1 persimmon fruits or achieved a 5-day commercial shelf life for the MS2 fruits. For the 1% CA-treated samples, Atm-E (5 kPa O_2) maintained good visual quality for 9 storage days at 5 ºC for both MSs. The fruits stored in either air (Atm-A) or 5 kPa O_2 + 10 kPa CO_2 (Atm-D) reached the limit of marketability by storage day 7 at 5 ºC in MS1, whereas those processed with MS2 were still marketable at the end of the study, regardless of the atmosphere tested. The shorter commercial shelf life of the persimmon slices placed in Atm-D (5 kPa O_2 + 10 kPa CO_2), if compared to Atm-E (5 kPa O_2), can be attributed to the 'flesh browning' incidence in fruits which, despite not being as severe as in experiment 1 for the samples placed in Atm-B (air + 10 kPa CO_2) and Atm-C (air + 20 kPa CO_2), also affected the visual quality of the fresh-cut persimmons. Therefore, these results confirm that CO_2 accumulation in the packaging of fresh-cut 'Rojo Brillante' persimmons should be avoided in order to prolong their commercial shelf life. 'Flesh browning' incidence was also affected by MS, which was higher in the fruits with MS2. Recent studies done with whole 'Rojo Brillante' persimmons have also indicated that superoxide anion levels gradually increased with persimmon maturation after removal astringency (Novillo et al., 2014a). Similarly, oxidative stress associated with fruit ripening has also been reported in other species, such as mango (Singh and Dwivedi, 2008), peach (Camejo et al., 2010) or papaya (Couto et al., 2012).

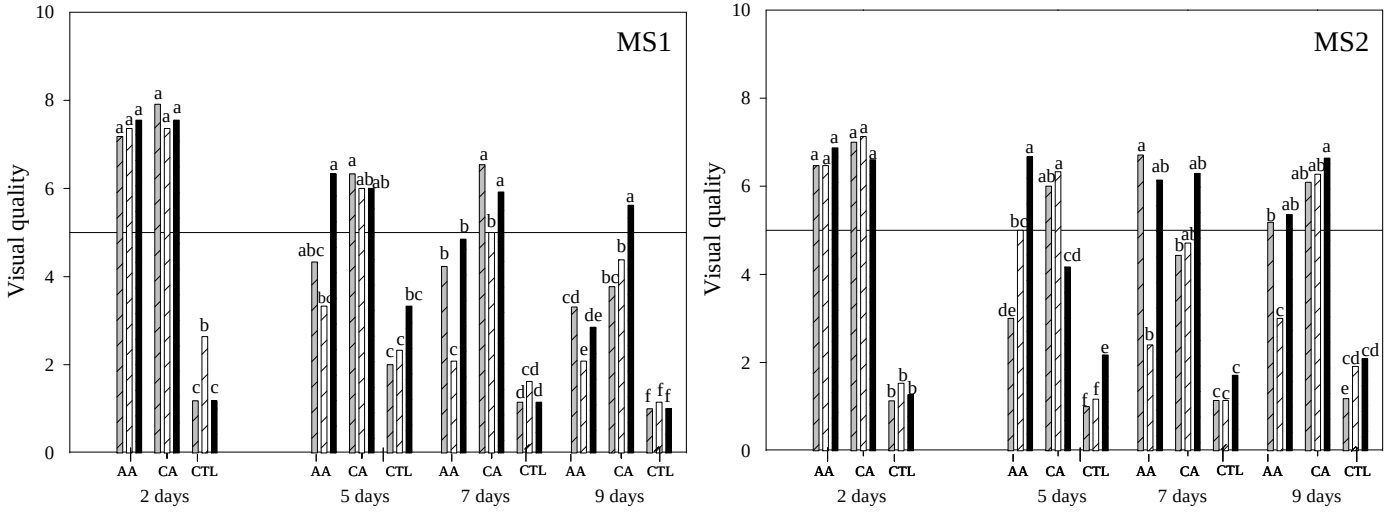

Fig. 6. Visual quality of fresh-cut 'Rojo Brillante' persimmons stored in atmospheres Atm-A ( ), Atm-D ( ) or Atm-E ( ) Persimmons were processed in two maturity stages (MS1 and MS2). Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2. The results are average values. Bars with different letters are significantly different at the 95% level.

Bioactive compounds

Fruit stage at harvest is one of the major factors that affects the nutritional value of fruits. Prolonged maturity may increase, decrease or have no effect on specific nutritional compounds, depending on the compound, and on the species or cultivars. In this work, the fruits processed in MS2 obtained higher values for TVC and total carotenoids, but lower values for TPC and radical scavenging activity than those processed in MS1 (Table 1, Table 2). Similar trends in TVC, radical scavenging activity and total carotenoids have been observed for fresh-cut 'Rojo Brillante' as being affected by MS at harvest, but not for total phenolic content (Sanchís et al., 2015). In all cases, the concentrations obtained herein fell within the same range as those obtained in other studies for non astringent persimmon cultivars and astringent cultivar 'Rojo Brillante' at similar harvest periods (Del Bubba et al., 2009; Sanchís et al., 2015; Wright and Kader, 1997a). Processing, antioxidants dips and controlled atmosphere storage had no clear effect on the different bioactive compounds tested, and the differences observed between treatments could be attributed to biological variation. The results that reflect phytonutrient stability or the effectiveness of postharvest treatments on the nutritional value of minimally processed fruits and vegetables generally differ according to the fruit commodity and processing conditions. Some works have reported that antioxidant dips, such as ascorbic acid and CA, increase the level of ascorbic acid and help maintain the TPC of fresh-cut apples (Cocci et al., 2006), kiwifruits (Antunes et al., 2010) and mangoes (Robles-Sánchez et al., 2013; Siddiq et al., 2013) for 8-12 days at 4 ºC. However in recent works, no clear effect of antioxidant dips based on AA and/or CA on total vitamin C, and on the radical scavenging activity of fresh-cut 'Rojo Brillante' persimmon, was observed (Sanchís et al., 2015). The effects of modified and/or controlled atmospheres on the bioactive compounds of fresh-cut fruits and vegetables have not been extensively studied. The use of low O_2 atmospheres has been generally reported to reduce vitamin C loss by inhibiting its oxidation, whereas high CO_2 has been described to cause degradation by stimulating the oxidation of ascorbic acid to dehydroascorbic acid (Gil and Kader, 2008). Thus high O_2 or CO_2 concentrations induced more marked vitamin C losses in fresh-cut pears (Oms-Oliu et al., 2008) and strawberries (Odriozola-Serrano et al., 2010).

Table 1 Effect on controlled atmosphere storage at 5 ºC and antioxidant application on total vitamin c and free radical scavenging activity of sliced 'Rojo Brillante' persimmons.

			Total vitamin C (mgAA/100g)				Free radical scavenging activity (g /kg DPPH˙)			
Day	Atm		MS1		MS2		MS1		MS2	
0			183.3 ± 35.0	B	354.6 ± 40.4	A	253.9 ± 18.0	A	145.1 ± 17.3	B
2	A	1% AA	242.8 ± 62.0	aA	347.1 ± 14.1	A	113.1 ± 4.0	dB	177.3 ± 13.9	dA
		1% CA	184.9 ± 40.4	abA	191.8 ± 20.7	bcdA	318.8 ± 30.2	bcA	252.3 ± 35.9	cA
		CTL	192.9 ± 18.3	abA	120.0 ± 17.0	dB	367.0 ± 35.7	abA	333.0 ± 16.0	bA
	D	1% AA	167.8 ± 31.6	abA	177.3 ± 31.8	cdA	277.0 ± 12.6	cB	403.9 ± 23.6	aA
		1% CA	135.0 ± 17.2	bA	150.3 ± 19.2	cdA	438.5 ± 23.9	aA	242.3 ± 16.2	cB
		CTL	182.7 ± 30.5	abA	247.0 ± 26.9	abcA	442.6 ± 52.1	aA	250.9 ± 27.3	cB
	E	1% AA	192.7 ± 27.7	abA	317.5 ± 69.8	aA	374.2 ± 15.7	abA	418.7 ± 17.8	aA
		1% CA	128.2 ± 9.4	bA	189.0 ± 40.0	bcdA	355.4 ± 18.6	bcA	176.4 ± 5.9	dB
		CTL	164.7 ± 23.7	abA	301.3 ± 80.2	abA	388.9 ± 27.8	abA	161.1 ± 12.1	dB
5	A	1% AA	162.8 ± 30.2	abA	212.1 ± 31.0	cdeA	307.9 ± 48.0	aA	271.4 ± 16.3	bA
		1% CA	149.5 ± 17.4	bA	275.2 ± 71.8	bcdeA	294.8 ± 18.1	aB	429.5 ± 51.3	aA
		CTL	228.9 ± 28.6	aA	137.2 ± 10.5	eB	323.2 ± 22.3	aA	317.6 ± 25.7	bA
	D	1% AA	192.4 ± 46.3	abA	147.6 ± 11.5	deA	302.8 ± 36.1	aA	273.9 ± 13.9	bA
		1% CA	190.7 ± 12.3	abA	427.8 ± 57.9	aA	299.1 ± 20.9	aB	465.1 ± 49.5	aA
		CTL	129.8 ± 16.2	bB	293.1 ± 49.1	abcA	340.7 ± 16.3	aA	408.0 ± 37.6	aA
	E	1% AA	147.7 ± 11.7	bB	405.8 ± 51.0	abA	151.6 ± 20.3	bB	274.4 ± 21.0	bA
		1% CA	233.9 ± 38.4	aA	267.5 ± 65.4	bcdeA	159.5 ± 13.1	bB	215.4 ± 5.2	bA
		CTL	194.0 ± 27.5	abA	280.9 ± 59.4	bcdA	292.9 ± 5.9	aA	224.3 ± 11.3	bB
9	A	1% AA	158.4 ± 17.7	abB	257.9 ± 38.3	abA	332.8 ± 28.5	bcA	130.2 ± 7.9	fB
		1% CA	165.7 ± 60.8	abA	183.2 ± 27.0	bA	232.6 ± 20.8	dB	484.4 ± 35.7	aA
		CTL	160.5 ± 19.8	abA	277.7 ± 62.0	abA	379.7 ± 22.2	abA	393.3 ± 19.4	bA
	D	1% AA	107.1 ± 38.5	abB	224.8 ± 20.2	abA	383.6 ± 19.4	abA	300.9 ± 21.8	cB
		1% CA	86.6 ± 16.4	bB	286.1 ± 52.2	abA	230.4 ± 23.0	dB	388.9 ± 23.2	bA
		CTL	91.1 ± 11.6	bB	272.8 ± 32.7	abA	232.7 ± 15.3	dA	202.4 ± 26.1	deA
	E	1% AA	133.9 ± 28.2	abB	301.8 ± 40.4	aA	355.6 ± 23.1	abcA	170.4 ± 9.0	efB
		1% CA	120.0 ± 11.5	abA	191.7 ± 28.0	bA	409.0 ± 30.3	aA	211.5 ± 20.5	deB
		CTL	198.6 ± 15.1	aA	219.1 ± 8.7	abA	288.8 ± 8.2	cdA	238.1 ± 28.5	cdA

Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2.
Values are mean ± standard error
Small letters show significant differences among treatments within each storage time by the LSD test ($p \leq 0.05$).
Capital letters show significant differences between MSs by the LSD test ($p \leq 0.05$).

Table 2 Effect on controlled atmosphere storage at 5 ºC and antioxidant application on total phenolic content and total carotenoids of sliced 'Rojo Brillante' persimmons.

			Total phenolic content (mg GA/100g)		Total carotenoids (µg /100g)	
Day	Atm		MS1	MS2	MS1	MS2
0			8.9 ± 0.4 A	5.3 ± 0.4 B	189.4 ± 63.9 A	284.4 ± 10.3 B
2	A	1% AA	7.2 ± 0.5 abA	6.3 ± 0.5 aA	200.7 ± 42.2 bcA	256.9 ± 74.3 abA
		1% CA	7.2 ± 0.5 abA	6.4 ± 0.5 aA	165.6 ±15.3 cA	160.4 ± 0.2 bA
		CTL	8.8 ± 0.6 aA	7.3 ± 0.6 aA	226.3 ± 88.2 bA	326.9 ± 87.9 aA
	D	1% AA	8.2 ± 0.5 abA	7.3 ± 0.6 aA	185.7 ± 32.0 cA	203.8 ± 53.3 abA
		1% CA	7.8 ± 0.6 abA	7.5 ± 0.6 aA	144.6 ± 14.9 cB	220.5 ± 3.0 abA
		CTL	7.2 ± 0.5 abA	7.1 ± 0.6 aA	222.3 ± 35.9 bcA	261.7 ± 26.4 abA
	E	1% AA	7.7 ± 0.6 abA	6.6 ± 0.6 aA	168.7 ± 11.7 cA	227.6 ± 12.6 abA
		1% CA	7.4 ± 0.7 abA	7.2 ± 0.5 aA	130.3 ± 5.9 cA	152.4 ± 21.9 bA
		CTL	6.8 ± 0.6 bA	7.3 ± 0.6 aA	333.5 ± 18.5 aA	251.1 ± 12.6 abB
5	A	1% AA	7.2 ± 0.6 bcdA	5.1 ± 0.4 cB	185.1 ± 42.4 abA	280.4 ± 29.5 aA
		1% CA	7.2 ± 0.5 cdA	7.5 ± 0.4 aA	200.3 ± 0.3 abA	295.8 ± 41.8 aA
		CTL	6.5 ± 0.4 deA	7.3 ± 0.6 abA	262.4 ± 11.6 aA	262.7 ± 28.4 aA
	D	1% AA	6.6 ± 0.5 deA	7.8 ± 0.6 aA	169.4 ± 7.6 abA	256.9 ± 58.4 aA
		1% CA	8.9 ± 0.5 aA	8.1 ± 0.6 aA	117.6 ± 67.0 bA	257.4 ± 23.1 aA
		CTL	8.1 ± 0.6 abcA	6.0 ± 0.5 bcB	118.2 ± 69.8 bA	267.0 ± 10.3 aA
	E	1% AA	8.7 ± 0.6 abA	7.0 ± 0.6 abA	230.6 ± 72.3 abA	197.1 ± 21.1 aA
		1% CA	7.0 ± 0.5 cdeA	5.4 ± 0.4 cB	111.6 ± 11.4 bB	302.8 ± 13.9 aA
		CTL	5.6 ± 0.4 eA	6.0 ± 0.5 bcA	186.2 ± 0.4 abA	305.5 ± 53.9 aA
9	A	1% AA	8.3 ± 0.5 aA	6.2 ± 0.4 cdeB	120.7 ± 8.5 abB	275.4 ± 21.6 bcA
		1% CA	5.3 ± 0.2 dB	7.1 ± 0.4 bcdA	152.3 ± 136.6 aA	292.7 ± 34.2 bcA
		CTL	7.1 ± 0.5 abcA	7.9 ± 0.4 abA	354.6 ± 0.7 bA	215.6 ± 0.3 bcB
	D	1% AA	7.4 ± 0.6 abcA	8.4 ± 0.4 aA	260.7 ± 63.3 aA	223.6 ± 6.5 cA
		1% CA	6.9 ± 0.5 bcA	6.5 ± 0.4 cdeA	233.8 ± 6.9 aB	431.5 ± 0.5 aA
		CTL	6.5 ± 0.5 cdA	7.1 ± 0.5 bcA	161.3 ± 44.1 abA	343.2 ± 3.0 abA
	E	1% AA	8.0 ± 0.4 abA	5.7 ± 0.5 eB	187.2 ± 50.2 abA	236.6 ± 27.0 cA
		1% CA	6.9 ± 0.4 bcA	5.4 ± 0.5 deA	213.9 ± 17.7 abA	272.9 ± 80.7 bcA
		CTL	7.8 ± 0.3 abA	5.8 ± 0.5 eB	249.2 ± 2.6 aA	321.2 ± 22.9 bcA

Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2.
Values are mean ± standard error
Small letters show significant differences among treatments within each storage time by the LSD test ($p \leq 0.05$).
Capital letters show significant differences between MSs by the LSD test ($p \leq 0.05$).

However in fresh-cut 'Fuyu' persimmons, storage in low O_2 (2 kPa) and/or high CO_2 (12 kPa) controlled atmospheres had no significant effect on the changes noted in total ascorbic acid content (Wright and Kader, 1997a).

Individual carotenoids were also analyzed and the results are shown in Fig. 7. The major carotenoids detected were β-cryptoxanthin and β-carotene. Although some significant differences were found among treatments, the results were variable, which makes it difficult to conclude the effectiveness of atmosphere conditions and antioxidant dips on these carotenoids in fresh-cut persimmons. Only the control samples with MS1 stored in air (Atm-A) presented higher concentrations of both carotenoids at the end of the 9-day storage. After 8 storage days at 5 ºC, Wright and Kader (1997b) reported a drop in the individual carotenoids content in 'Fuyu' persimmon slices stored in both 2 kPa O_2 and air + 12 kPa CO_2, but this loss was not that significant for the slices stored under the 2 kPa O_2 + 12 kPa CO_2 conditions. Overall, no significant losses in provitamin A were seen before the slices reached their limit of marketability.

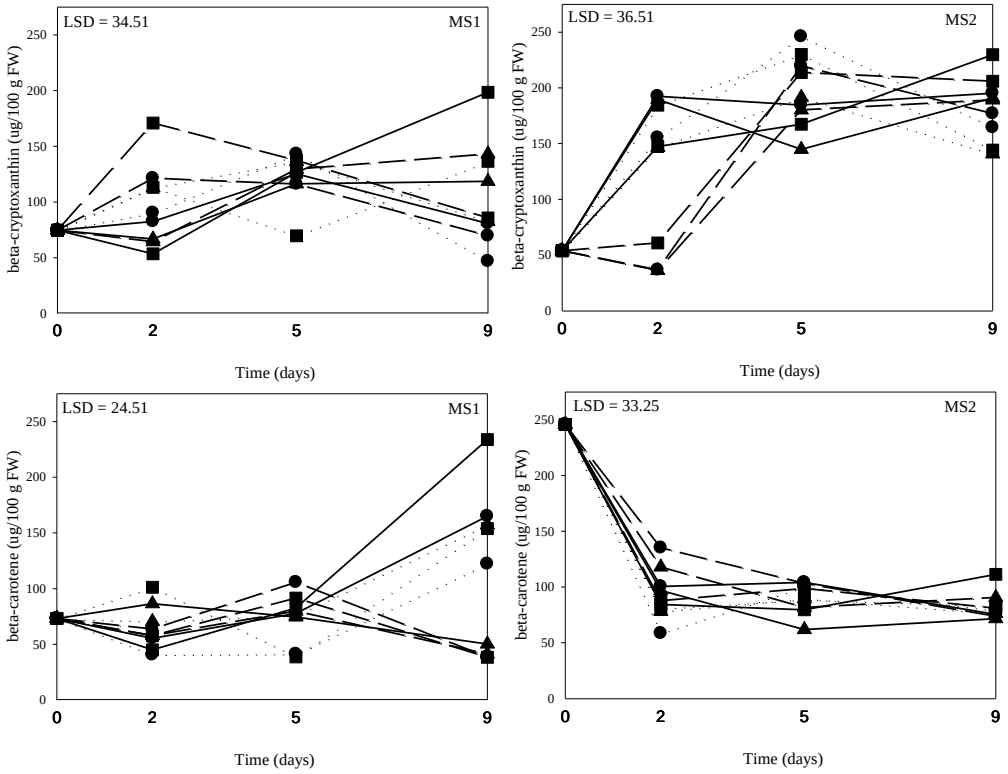

Fig. 7. β-cryptoxanthin and β-carotene content of fresh-cut 'Rojo Brillante' persimmons stored in controlled atmospheres Atm-A (——), Atm-D (– – –) or Atm-E (·····) and dipped in 1% AA (▲), 1% CA (●) or water as a control (■). Persimmons were processed in two maturity stages (MS1 and MS2). Atm-A = air; Atm-D = 5 kPa O_2 + 10 kPa CO_2; Atm-E = 5 KPa O_2. The results are average values.

4. Conclusion

The combination of high O_2 (21 kPa) and elevated CO_2 (10 or 20 kPa) does not prevent enzymatic browning and softening of fresh-cut 'Rojo Brillante' persimmons, and high CO_2 concentrations induce 'flesh browning' on tissue. Antioxidants and Atm-E (5 KPa O_2) proved to be most effective combination to prevent enzymatic browning and to maintain visual quality above the limit of marketability for 9 days at 5 ºC for both the maturity stages studied. TVC, free radical scavenging activity, TPC and carotenoid content were affected by the MS at harvest, whereas processing, antioxidants dips and controlled atmosphere storage had no clear effect.

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Chapter 5

Quality changes in fresh-cut 'Rojo Brillante' persimmons packed in modified atmosphere and dipped in antibrowning agents

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Abstract

Fresh-cut fruits, such as persimmons, have a very limited shelf life due to enzymatic browning. This work aims to evaluate the effect of antioxidant dip and modified atmosphere packaging (MAP) on the shelf life of fresh-cut 'Rojo Brillante' persimmons during storage at 5 ºC. Persimmon slices were dipped in an antioxidant solution (1% citric acid (CA) plus 1% calcium chloride ($CaCl_2$)) or water (control), and were packed in 5 kPa O_2 or air to achieve active or passive MAP, respectively. The MAP conditions in the headspace atmosphere did not overpass 10 kPa CO_2 and the O_2 concentrations fell within the 0.6-2.3 kPa range, depending on the initial gas composition. These values were within an acceptable range as they did not negatively affect the sensory quality of samples. The antioxidant dip reduced the enzymatic browning of persimmon slices, which correlated with lower polyphenoloxidase activity. By storage day 9, the antioxidant-treated samples packed in active MAP were evaluated as having 'very good' visual quality, whereas those in passive MAP were evaluated on the 'limit of marketability', which indicates a synergic effect of antioxidant and active MAP.

Keywords: Minimally processed persimmon; Citric acid; Calcium chloride; Shelf life; Sensory quality

1. Introduction

Persimmon fruit (*Diospyros kaki* L. Rojo Brillante) is an important cultivar in the Ribera del Xúquer region (Valencia, Spain) which, upon removal of astringency with high CO_2 concentrations, has the huge potential to become a high quality fresh-cut commodity. This cultivar is very much appreciated by consumers for its color, size, flavor and nutritional value, and is nowadays a real alternative to other Mediterranean crops, such as citrus fruits. However as with most fresh-cut fruits, the highest hurdle for commercial fresh-cut 'Rojo Brillante' persimmons marketing to overcome is their limited shelf life given their susceptibility to enzymatic browning.

The main approach to reduce the enzymatic browning of fresh-cut tissue is to use antibrowning agents and/or modified atmosphere packaging (MAP)

in synergy with proper temperature management. Previous works by our group have shown the beneficial effect of acidic solutions, such as citric and ascorbic acid, in combination with $CaCl_2$, to control enzymatic browning and to maintain firmness loss of fresh-cut 'Rojo Brillante' persimmons (Sanchís et al. 2015a; 2015b). Further studies have evaluated the effect of different controlled atmospheric conditions and antioxidant dips as a first step to select optimum O_2 and CO_2 concentrations for the MAP of fresh-cut 'Rojo Brillante' persimmons. Overall, the combination of antioxidant dips and a controlled atmosphere, composed of 5 kPa O_2 (balance N_2), has proved to be the most effective combination that prevents enzymatic browning, and also for visual quality, which reached the limit of marketability by storage day 9 at 5 ºC (Sanchís et al. 2015c). Beyond these works, there are no published reports about extending the shelf life of fresh-cut persimmons that have used combinations of $CaCl_2$, citric acid and MAP. Therefore, the aim of this work was to study the effect of passive and active MAP (5 kPa O_2) on the color, firmness and sensory quality of minimally processed 'Rojo Brillante' persimmons dipped in an antioxidant solution during storage at 5 ºC.

2. Materials and methods

Fresh persimmon fruits (Diospyros kaki cv. Rojo Brillante) were provided by the Protected Designation of Origin (PDO) Kaki Ribera del Xúquer (Valencia, Spain). Persimmon fruits were harvested in a commercial maturity stage with flesh firmness of 33±6 N, a soluble solid content of 15.1±0.2 ºBrix, and total acidity of 0.47±0.03 g malic acid L^{-1}. Immediately, astringency was removed by the application of 95% of CO_2 in closed chambers for 24 h at 20º C and 90% relative humidity (RH). The non astringent persimmons, pre-cooled at 5 ºC, were washed with chlorinated water (150 mg L^{-1}), peeled, and cut into eight wedges with a sharp stainless steel knife. Half the slices were immersed into an antioxidant solution of 1% (w/v) citric acid (CA) (Quimivita S.A., Spain) plus 1% (w/v) calcium chloride ($CaCl_2$) (antioxidant dip) (Sigma-Aldrich, St. Louis, MO, USA), while the other half were immersed in water (control) for 3 min. After draining and drying under cold conditions, four pieces (115±10 g) were placed on polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain). Trays were heat-sealed with a 64-μm thickness PP/PET film that had an oxygen and carbon dioxide transmission rates of 110 and

500 mL/m^2/24h/bar, respectively (P12-2050PXNP, ILPRA Systems España S.L. Mataró, Spain). The MAP conditions of the antioxidant dipped and control samples were achieved by flushing trays with 5 kPa O_2 (balanced with N_2) (active MAP) or by conventional storage in air using the same film (passive MAP). The fresh-cut persimmons were stored at 5 ºC for 9 days. Five replicate trays per treatment were used on each storage day.
The gas composition in the package headspace was analyzed in a gas chromatograph (Trace GC, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector (TCD). The oven, injector and TCD temperatures were 35, 115, and 150 ºC, respectively. One mL of the gas headspace was injected into the system. Measurements were taken on five trays per treatment.

The color of the persimmons pieces was determined with a Minolta CR-400 chromameter (Konica Minolta Sensing, Inc., Osaka, Japan) on 12 pieces per treatment using CIELAB color parameters L* and a*. All the measurements were taken at three locations on each sample piece.

Polyphenol oxidase (PPO) activity was determined by adding 3 mL of 0.05 M 4-methylcatechol to 100 μL of the enzyme extract. This extract was obtained by mixing 15 g of fresh persimmon with McIlvaine buffer solution (1:1) at pH 6.5, which contained 1 M sodium chloride and 5 % polyvinylpolypyrrolidone. The homogenate was centrifuged at 12000 rpm at 4 ºC for 30 minutes. Changes in absorbance were determined at 420 nm every 5 sec up to 2 min in a spectrophotometer (UV-1, Thermo Electron Corporation, UK) and activities were expressed in absorbance per minute. Each sample was measured in duplicate. All the reagents used were obtained from Sigma-Aldrich (St. Louis, MO, USA).

The firmness of the fresh-cut persimmons 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 and at a speed of 5 mm/s. Twelve samples per treatment were measured. The results are expressed in newtons (N).
The sensory evaluation was made by 15 trained judges, and included visual and taste assessments. General visual quality was evaluated according to 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. A color photograph of the samples rated with this scale was used by the judges

to score the samples. Each treatment was presented to the judges on trays labeled with a 3-digit random code and contained 12 persimmon pieces to account for sample variability. The effect of treatments on taste included characteristic 'persimmon' flavor, off-flavor, firmness and overall quality. Characteristic flavor and off-flavor were rated on a 5-point scale, where 1 = no presence and 5 = marked presence. Firmness was rated on a 5-point scale, where 1 = very soft and 5 = very firm. Overall quality was rated on a 9-point scale, where 1 represented very poor quality and 9 denoted excellent quality. Two persimmon slices randomly selected from each treatment were presented to the judges on trays labeled with 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 color, samples were illuminated with appropriate lighting to completely mask browning.

Statistical analyses were performed with STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences among treatments were determined by least significant differences (LSD) when the analysis of variance (ANOVA) showed a significant F-value. Significant differences were defined at $p \leq 0.05$.

3. Results and discussion

The O_2 concentration in the active and passive MAP reached equilibrium by storage day 4, with values of 0.6 kPa and 2.3 kPa, respectively (Fig. 1). In contrast, a steady increase in CO_2 was seen in the headspace atmosphere during the 9 storage days under both MAP conditions, with values between 6.8 and 9.8 kPa. The antioxidant dip increased the CO_2 concentration on the trays compared to the control for both MAP conditions, which indicates an increase in the respiration rate of fruits by the antioxidant treatment. Overall, the highest CO_2 value was reached in the antioxidant-dipped persimmons stored in passive MAP. Tables with accepted optimal atmospheres and handling temperatures for some fresh-cut fruits and vegetables have been published by Gorny (2003). In these tables, the atmosphere recommended for sliced persimmons stored at 0-5 ºC is 2 kPa O_2 and 12 kPa CO_2. However, caution must be taken in applying the recommended optimal atmosphere for a given commodity since there may be varying responses by a product in accordance with factors like cultivar, physiological maturity, growing conditions, postharvest

handling conditions, etc. (Toivonen et al. 2009). In a previous work by our group conducted with fresh-cut 'Rojo Brillante' persimmons, the application of controlled atmospheres with high CO_2 concentrations (10 or 20 kPa) induced a physiological disorder on the tissue known as 'the flesh browning disorder', which negatively affected the visual quality of the samples (Sanchís et al. 2015c). In the present work, the atmospheres obtained in packaging by storage day 9 did not overpass 10 kPa CO_2. Yet despite the O_2 concentrations being low, they fell within an acceptable range, as reported in the sensory evaluation of the samples

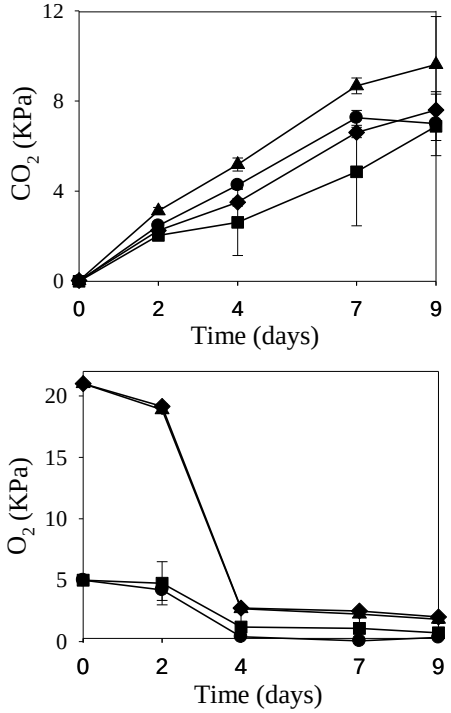

Fig. 1 Effect of antioxidant dip and modified atmosphere packaging (MAP) on oxygen and carbon dioxide of fresh-cut 'Rojo Brillante' persimmons during storage at 5 ºC. (●) antioxidant dip-active MAP; (■) control-active MAP; (▲) antioxidant dip-passive MAP; (♦) control-passive MAP. Active MAP (5 kPa O_2, balance N_2); passive MAP (air). Antioxidant dip (1% citric acid + 1% $CaCl_2$); Control (water). Vertical bars are standard error.

The use of active MAP significantly reduced the enzymatic browning of the control samples, which obtained lower *a** and higher *L** values than those packed under the passive MAP conditions (Fig. 2). The a* values of the tissue remained low when the antioxidant solution was applied, and no differences were observed between active and passive MAP. These results suggest that the rapid establishment of low O_2 in the packaging atmosphere (active MAP) can be an effective way to prevent the enzymatic browning of fresh-cut 'Rojo Brillante' persimmons when no antioxidant treatment is applied. However, the application of active or passive MAP to antioxidant-treated persimmons is less critical for reducing surface browning, which indicates that the tested antioxidants exerted proper enzymatic browning control. The effectiveness of CA and $CaCl_2$, either alone or in combination, to reduce enzymatic browning has also been reported in fresh-cut 'Rojo Brillante' persimmons (Sanchís et al. 2015a; 2015b) and other fresh-cut fruits like apples (Rocculi et al. 2004) or papayas (Waghmare and Annapure 2013). Some of these works have also shown a prolonged postharvest shelf life of fresh-cut fruits as the chemical treatment was combined with proper MAP conditions (Rocculi et al. 2004; Waghmare and Annapure 2013).

The effectiveness of an antioxidant dip to control browning correlated with the lower PPO activity of fresh-cut 'Rojo Brillante' persimmons compared to untreated samples (Fig. 3). This can be explained by the effect of CA lowering the pH below the optimum value for PPO activity. However, applying active MAP as a single treatment did not reduce PPO activity compared to passive MAP.

Persimmon slices presented a firmness loss of between 50% and 70% by storage day 4, and values were maintained thereafter (Fig. 4). Under both MAP conditions, the persimmon slices dipped in the antioxidant treatment obtained lower firmness values than the untreated samples. In previous studies, the application of acidic solutions, like citric or ascorbic acid, proved effective for preventing the enzymatic browning of fresh-cut persimmons, but led to major tissue softening if compared to the untreated samples (Sanchís et al. 2015a). Further studies have demonstrated that the combination of these antioxidants with 1% $CaCl_2$ helped to maintain the firmness of the persimmon slices, and within the same range as the control samples, and also compensated for the loss of firmness produced by the antioxidant (Sanchís et al. 2015b). Fruit firmness reduced by acid solutions

has also been reported in other fresh-cut fruits, like pears and apples, which have indicated some damage to cell wall structure (Oms-Oliu et al. 2006; Rojas-Graü et al. 2007). Yet the effect of active MAP to reduce firmness loss of fresh-cut persimmons was observed only in the control samples, whereas those dipped in antioxidant solution were not affected by packaging conditions.

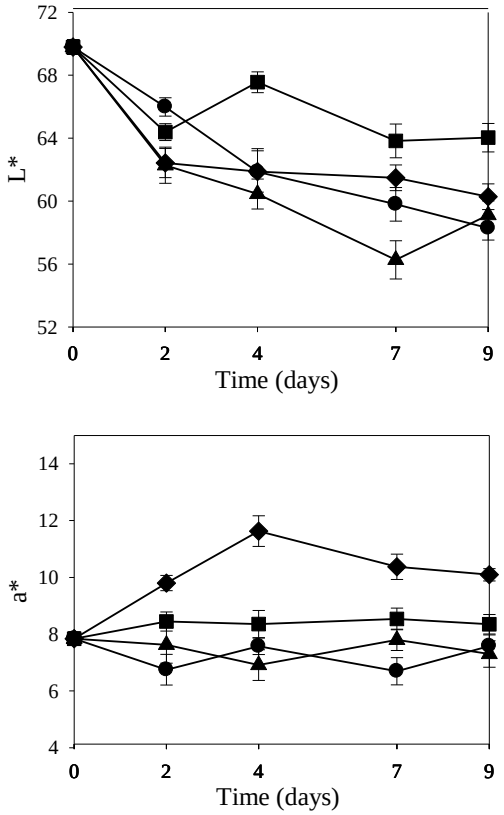

Fig. 2 Effect of antioxidant dip and modified atmosphere packaging (MAP) on colour *L** and *a** values of fresh-cut 'Rojo Brillante' persimmons stored at 5 ºC during 9 days. (●) antioxidant dip-active MAP; (■) control-active MAP; (▲) antioxidant dip-passive MAP; (♦) control-passive MAP. Active MAP (5 kPa O_2, balance N_2); passive MAP (air). Antioxidant dip (1% citric acid + 1% $CaCl_2$); Control (water). Vertical bars are standard error.

The commercial shelf life of fresh-cut persimmons was evaluated according to visual quality (Fig. 5). Only the samples dipped in antioxidant solution were evaluated above the limit of marketability during the 9 storage days at 5 ºC, whereas the control samples were evaluated below this limit by storage day 2. No differences were observed between the active and passive MAPs of the antioxidant-dipped samples during the first 4 days of storage. However after 9 storage days, the antioxidant-treated samples packed in active MAP (the 5kPa O_2 initial atmosphere) were still evaluated by the judges as having a 'very good' visual quality, while the samples in passive MAP were only on the 'limit of marketability'. These results indicate that although active MAP is not necessary to maintain the visual quality of treated persimmons within the limit of marketability for 9 days, it offers a synergic effect to improve visual quality.

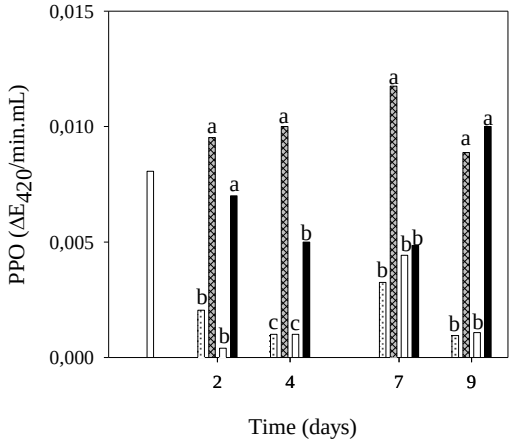

Fig. 3 Polyphenol oxidase (PPO) activity of fresh-cut 'Rojo brillante' persimmons dipped in antioxidant and stored in modified atmosphere packaging (MAP) at 5 ºC during 9 days. (▨) antioxidant dip-active MAP; (▩) control-active MAP; (▭) antioxidant dip-passive MAP; (▬) control-passive MAP. Active MAP (5 kPa O_2, balance N_2); passive MAP (air). Antioxidant dip (1% citric acid + 1% $CaCl_2$); Control (water). Results are average values. For each storage period, bars with different letter are significantly different at 95% level.

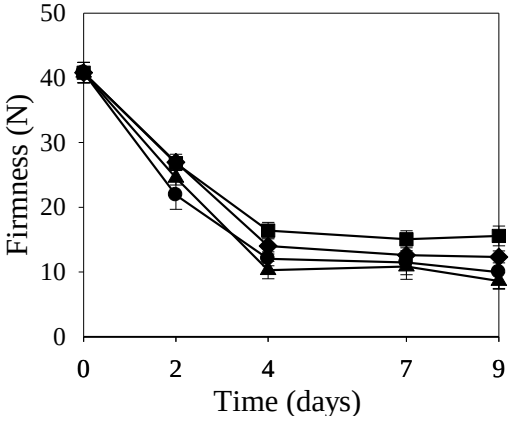

Fig. 4 Effect of antioxidant dip and modified atmosphere packaging (MAP) on firmness of fresh-cut 'Rojo Brillante' persimmons stored at 5 ºC during 9 days. (●) antioxidant dip-active MAP; (■) control-active MAP; (▲) antioxidant dip-passive MAP; (♦) control-passive MAP. Active MAP (5 kPa O_2, balance N_2); passive MAP (air). Antioxidant dip (1% citric acid + 1% $CaCl_2$); Control (water). Vertical bars are standard error.

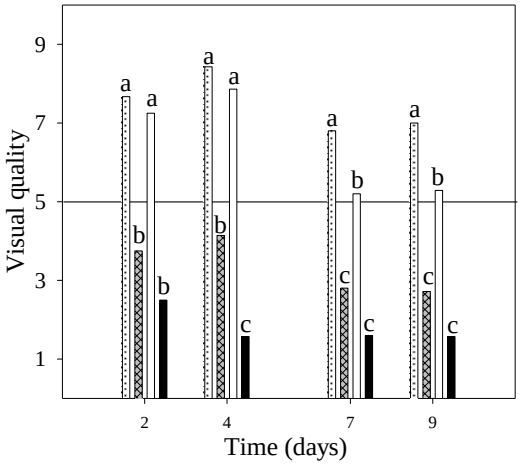

Fig. 5 Visual quality of fresh-cut 'Rojo brillante' persimmons dipped in antioxidant and stored in modified atmosphere packaging (MAP) at 5 ºC during 9 days. ( ) antioxidant dip-active MAP; ( ) control-active MAP; ( ) antioxidant dip-passive MAP; ( ) control-passive MAP. Active MAP (5 kPa O2, balance N2); passive MAP (air). For each storage period, bars with different letter are significantly different at 95% level.

The effect of treatments on fresh-cut persimmon taste was significant differences in the characteristic flavor of samples by storage day 2 (Table 1). These differences were attributed to the acidic taste of the antioxidant-treated samples, as reported by the judges in the additional comments they made. This was also reflected in a slight off-flavor or atypical flavor. These differences among treatments disappeared after 4 storage days and did not affect overall flavor, which fell within the range of acceptability throughout the storage period at 5 ºC. Persimmon slice firmness diminished with storage, but no differences were found among treatments, except at the end of the 9-day storage, when the antioxidant-treated samples were evaluated as being soft.

Table 1 Effect of modified atmosphere packaging (MAP) and antioxidant dip on the characteristic and overall flavor, off-flavors and sensory firmness of fresh-cut 'Rojo Brillante' persimmons during 9 storage days at 5 ºC.

Storage (days at 5ºC)	Treatment	Characteristic flavor	Off-flavor	Firmness	Overall Flavor
0		4.4 ± 0.9	1.0 ± 0.0	4.2 ± 0.5	7.8 ± 1.1
2	Antioxidant dip + active MAP	2.9 ± 1.0c	2.0 ± 0.8ab	3.9 ± 0.4a	5.6 ± 1.3a
	Control + active MAP	4.1 ± 0.6a	1.0 ± 0.0c	4.0 ± 0.8a	6.9 ± 0.6a
	Antioxidant dip + passive MAP	3.1 ± 1.2bc	2.1 ± 1.6a	3.6 ± 0.5a	5.8 ± 1.8a
	Control + passive MAP	3.9 ± 0.6ab	1.1 ± 0.4bc	3.8 ± 0.9a	6.9 ± 0.8a
4	Antioxidant dip + active MAP	2.5 ± 1.4a	2.0 ± 1.3a	3.5 ± 0.8a	5.2 ± 1.7a
	Control + active MAP	3.5 ± 1.2a	1.2 ± 0.4a	3.5 ± 0.8a	6.5 ± 1.6a
	Antioxidant dip + passive MAP	2.7 ± 1.0a	2.0 ± 1.1a	3.7 ± 0.5a	5.0 ± 1.4a
	Control + passive MAP	3.5 ± 1.1a	1.5 ± 0.8a	3.0 ± 1.1a	6.0 ± 1.3a
7	Antioxidant dip + active MAP	4.0 ± 0.8a	1.0 ± 0.0a	3.3 ± 1.0a	7.0 ± 0.8a
	Control + active MAP	4.0 ± 0.8a	1.0 ± 0.0a	3.8 ± 0.5a	6.8 ± 1.3a
	Antioxidant dip + passive MAP	3.8 ± 1.0a	1.0 ± 0.0a	3.5 ± 0.6a	6.5 ± 1.3a
	Control + passive MAP	4.2 ± 0.5a	1.0 ± 0.0a	3.8 ± 0.5a	7.3 ± 0.5a
9	Antioxidant dip + active MAP	3.4 ± 1.0a	1.4 ± 0.8a	2.6 ± 0.5b	5.9 ± 1.4a
	Control + active MAP	4.0 ± 0.6a	1.0 ± 0.0a	3.3 ± 0.5a	6.9 ± 0.9a
	Antioxidant dip + passive MAP	3.4 ± 0.8a	1.6 ± 0.8a	2.4 ± 0.5b	5.7 ± 1.1a
	Control + passive MAP	3.7 ± 1.1a	1.4 ± 0.8a	3.0 ± 0.8ab	6.3 ± 1.6a

Antioxidant dip = 1% citric acid + 1% $CaCl_2$; Control = water; Active MAP = 5 kPa O2, balance N2; Passive MAP =air.
Values are means ± standard deviation.
Values within the storage time followed by the same letter indicate that the mean values are not significantly different by the LSD test ($p \leq 0.05$).

4. Conclusion

The results indicate that the combination of 1% CA and 1% $CaCl_2$ as an antioxidant treatment controls tissue browning and maintains the general visual quality of fresh-cut persimmons within the limit of marketability for 9 storage days at 5 ºC. Although the combination of this antioxidant treatment and active MAP in 5kPa O_2 is not necessary to accomplish a 9-day commercial shelf life, helps improve the visual quality of fresh-cut 'Rojo Brillante' persimmons, and shows a synergic effect of antioxidant and active MAP.

Acknowledgements

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Chapter 6

Antioxidant edible coatings help maintain the physical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmons

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Abstract

Edible coatings were prepared from whey protein isolate (WPI), soy protein isolate (SPI), hydroxylpropyl methylcellulose (HPMC) and apple pectin as the hydrophilic phase, and beeswax (BW) as the lipid phase. As antioxidants, 10 g kg^{-1} citric acid (CA) and 10 g kg^{-1} $CaCl_2$ were added into formulations. Persimmon slices harvested in two maturity stages (MSs) 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. Colour, firmness, visual quality, overall sensory flavour and nutritional quality were measured during storage. Coated samples and those dipped in the antioxidant aqueous solution presented lower a^* and higher L^* values than the control samples, which indicated effective browning inhibition. Overall, the persimmon slices treated with the HPMC- and pectin-based coatings reached the limit of marketability after 9 storage days in the fruits harvested in the first half of the season. In late season persimmons, the limit of marketability was conditioned by the burst of a disorder known as 'flesh browning', which negatively affected the visual quality of the samples. Nutritional and flavour quality were not negatively affected by cutting, coating treatments or storage at 5 ºC.

Keyword: Whey protein isolate, soy protein isolate, hydroxylpropyl methylcellulose, apple pectin, antioxidants, shelf life.

INTRODUCTION

The demand for fresh-cut fruit and vegetables has continuously increased over the last few years, and the convenience factor and the healthy eating trend are the main reasons for growth. Among them, demand for bagged salads and cut vegetables has grown the most, and their market has consolidated well, whereas fresh-cut fruits have grown at a slower rate given their higher perishability. Fresh-cut fruits on today's market mainly include melon, cantaloupe, watermelon, grapefruit, pineapple and grape, and their fruit mixes. Therefore, the development of new good quality fruit products is both a challenge and a marketing opportunity for the food industry. In recent years, persimmon (*Diospyros kaki* L.) 'Rojo Brillante' production has

significantly increased in the 'Ribera del Xúquer' area (Valencia, Spain), and this fruit is now considered an alternative to other Mediterranean crops such as citrus. When harvested it is an astringent variety, but the application of high CO_2 concentrations allows its commercialisation as a non-astringent fruit without affecting its firmness. This cultivar is characterised by its excellent colour, size, flavour and nutritional value, which make it a good candidate to be prepared as a fresh-cut commodity. Yet the future of this commodity as a fresh-cut product depends on the development of proper post-processing technologies to help preserve its fresh-like quality over prolonged periods. Recent studies conducted by our group have shown the beneficial effect of some antioxidants, such as citric and ascorbic acid, to control the enzymatic browning of fresh-cut 'Rojo Brillante' persimmons and the importance of the maturity stage (MS) at harvest for the commercial shelf life of fresh-cut 'Rojo Brillante' persimmons (Sanchís et al., 2015).

Another approach to extend the shelf life of fresh-cut fruit and vegetables that has gained plenty of attention in the last decade is the use of edible coatings. Such coatings can provide a semipermeable barrier to gases and water vapour, which might translate in a reduction in respiration rate, as well as less enzymatic browning and water loss (Pérez-Gago et al., 2005a). The development of edible films and coatings has focused on proteins such as whey protein, casein or soy protein, polysaccharides such as chitosan, cellulose, pectin, alginate, carrageenan, and starch, and lipids such as fatty acids, beeswax and carnauba. Among then, hydroxypropyl methylcellulose (HPMC), pectin, whey protein isolate (WPI) and soy protein isolate (SPI) have shown good film-forming properties, and the capability of yielding tough flexible transparent films with good oxygen barrier properties at low relative humidity (RH) (Krochta, 1996). Several studies have also shown their potential to extend the shelf life of some fresh-cut fruits, whose properties have improved by adding antioxidant agents to the formulations. Thus, HPMC and WPI-based coatings reduced the enzymatic browning of fresh-cut apples (Pérez-Gago et al., 2005a), SPI-based coatings effectively controlled the browning of fresh-cut potatos, apples, eggplants and persimmons (Shon and Haque, 2007; Ghidelli et al., 2010a, b), and pectin-based coatings were effective for avoiding the browning of, and for preserving the vitamin C and phenolic content, in fresh-cut pears (Oms-Oliu et al., 2008).

The effectiveness of edible coatings for preserving the quality of fresh-cut products may vary depending on the composition of the coating, the commodity type, variety and maturity, the degree of surface coverage, and storage conditions (González-Aguilar et al., 2010). Pérez-Gago et al. (2005a) showed that selecting the hydrophilic component is important in the formulation of coatings for fresh-cut products. Their results revealed that whey protein-based coatings were more effective for controlling the enzymatic browning of fresh-cut apples than HPMC-based coatings, probably due to the antioxidant effect of some amino acids, such as cysteine, which are present in protein, or to the higher oxygen barrier that this coating exerts. Similarly, fresh-cut melons coated with pectin maintained better quality attributes compared to the samples coated with gellan or alginate after 1 week of storage (Oms-Oliu et al., 2008). Therefore, the aim of this study was to evaluate the effect of different antioxidant edible coatings prepared from HPMC, WPI, SPI and pectin on the physico-chemical, sensory and nutritional quality of fresh-cut 'Rojo Brillante' persimmons harvested in two commercial MSs.

MATERIAL AND METHODS

2.1. Coating materials and reagents

Soy protein isolate (SPI) (Supro 760IP) was supplied by Solae (Ieper, Belgium) and whey protein isolate (WPI) was purchased from Davisco Foods International (Le Sueur, MN, USA). Hydroxypropyl methylcellulose (HPMC) (Methocel El5) was supplied by Dow Chemical Co. (Midland, MI, USA) and apple pectin was acquired from Sigma-Aldrich (St. Louis, MO, USA). Beeswax (BW) (Brillocera S.A., Valencia, Spain) was selected as the lipid phase of the different protein and polysaccharide edible coatings. Glycerol (Panreac Quimica, Barcelona, Spain) was used as a plasticizer. Citric acid (CA) was supplied from Quimivita (Barcelona, Spain) and calcium chloride ($CaCl_2$) from Sigma-Aldrich (St. Louis, MO, USA). The reagents for the analysis of bioactive compounds included 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, sodium chloride, ammonium acetate, β-apo-8'-carotenal and β-carotene, which were purchased from Sigma-Aldrich (St. Louis, MO, USA); methanol, chlorhydric acid, ethanol, hexane, methylene chloride, acetonitrile and butylated hidroxytoluene from Scharlau (Barcelona, Spain); β-

cryptoxanthin, lutein, lycopene and zeaxanthin, which came from Extrasynthese (Genay, France); gallic acid was acquired from Acros Organics (Geel-Belgium) and thriethylamine came from Panreac (Barcelona, Spain). All the solvents used for the analysis of bioactive compounds were HPLC-grade and Milli-Q system ultra-pure water (Millipore Corp., USA) was used.

2.2. Coating formulations

Emulsion coatings were prepared by first dissolving the different polymers in distilled water. The aqueous solution of HPMC (30 g kg^{-1}) was dispersed in hot water at 80 ºC and was later hydrated at 20 ºC for 30 min. The apple pectin solution was prepared by dissolving 20 g kg^{-1} pectin in water at 25 ºC by mild stirring. To prepare the SPI and WPI emulsion coatings, aqueous solutions of 30 g kg^{-1} protein were prepared and heated for 30 min in a water bath at 90 ºC to denature proteins. BW was added to the polysaccharide or protein aqueous solutions at a concentration of 15 g kg^{-1}. In all the coating formulations, glycerol was added as plasticizer at a polymer (polysaccharide or protein) to glycerol ratio of 2:1. To help melt the BW, solutions were heated to 10-20 ºC above the melting point of BW. Once the lipid was melted, samples were homogenised in a high-shear probe mixer (PolyTron, ModelPT2100; Kinematica AGInc., Lucerne, Switzerland) for 4 min at 30,000 rpm. After homogenisation, emulsions were cooled in an ice bath to crystallise the lipid particle. Finally, antioxidants (10 g kg^{-1} CA plus 10 g kg^{-1} $CaCl_2$) were incorporated into each emulsion coating by magnetic agitation. The WPI-, SPI- and HPMC-based emulsions were prepared with a total solid content of 8%, and the pectin-based emulsion with a total solid content of 6.5%. Emulsions were kept at 5 ºC until their application.

2.3. Fruit coating

Persimmon fruits (*Diospyros kaki* cv. Rojo Brillante) were provided by the Protected Designation of Origin (PDO) Ribera del Xúquer (Valencia, Spain) in two different commercial maturity stages (MSs) determined by external colour as MS1 with a colour index (CI) of -1.7±2.1 and MS2 with a CI of 14.2±2.7, where CI=1,000 *a/Lb*. These MSs were from fruits harvested in early October and mid-November, which respectively

corresponded to the beginning and the end of the season. Initial fruit firmness was 69.6±6.5 N and 40.6±4.5 N for MS1 and MS2, respectively.

Before processing, natural astringency was removed by applying 95±2 kPa of CO_2 in closed chambers for 24 h at 20 ºC and at 90% RH. The CO_2 level, temperature and RH in the chambers were continuously monitored by a computer-controlled system (Control-Tec®, Tecnidex S.A., Paterna, Valencia, Spain). After being removed from the chambers, fruits were pre-cooled at 5±1 ºC for 20 h, sanitised in a 150 mg L^{-1} NaClO solution for 2 min, rinsed with tap water and dried. Persimmon fruits were peeled, cut into eight wedges and dipped into the coating solutions for 3 min. As controls, fruit wedges were dipped in water or in the aqueous antioxidant solution (10 g kg^{-1} CA + 10 g kg^{-1} $CaCl_2$) under similar conditions. After draining and drying at 5±1 ºC, four persimmon pieces (115±10 g) were placed onto polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and sealed with microperforated polypropylene film (64-µm thickness; ILPRA Systems) as secondary packaging. To ensure that the surrounding atmosphere on the tray remained unchanged, and to study only the effect of the coating treatments, 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 day to determine colour, sensory and nutritional quality. Samples were stored up to 9 days at 5±1 ºC. The whole process was carried out in a temperature-controlled room at 5±1 ºC under suitable hygienic conditions.

2.4. 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) on 12 persimmon pieces per treatment. Each measurement was taken randomly at three different locations per sample piece. A standard white calibration plate was employed to calibrate the equipment. The results were expressed as the means of 12 samples per treatment.

2.5. Firmness measurements

The firmness of fresh-cut persimmons 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 and at a speed of 5 mm/s. Twelve samples per treatment were measured and the results were expressed in newtons (N).

2.6. Sensory quality

The sensory quality of persimmon slices was conducted by 15 trained judges, and included a visual and taste evaluation. For the visual test, each treatment was presented to the panelists on trays that contained 12 persimmon pieces to account for sample variability, which were labelled with a 3-digit random code and presented to the judges under the same conditions (light intensity and temperature) to minimise variations in human perception. 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 the samples.

The taste evaluation included the overall flavour, off-flavours, and firmness of the fresh-cut persimmons. Overall flavour was rated on a 9-point scale, where 1 represented a very poor flavour and 9 an excellent flavour. 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, 3 = neither firm nor soft, and 5 = very firm. Two persimmon slices, randomly selected from each treatment to compensate for the biological variation of the material, were presented to the panelists on trays labelled with 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.

2.7. Bioactive compounds

Total vitamin C (TVC), free radical scavenging activity, total phenolic content (TPC) and carotenoids were evaluated in the fresh-cut ‘Rojo Brillante’ persimmons processed in two different MSs and stored for 2, 5

and 9 days at 5 ºC. On each sampling day, 12 persimmon slices per treatment were frozen in liquid nitrogen and kept at -80 ºC until analysed. Bioactive compounds were determined in three replicates per treatment.

TVC was determined as described by Wright and Kader (1997a) as the sum of ascorbic acid and L-dehydroascorbic acid. Two grams of persimmon samples were homogenised with 38 mL of a solution of 0.1 M citric acid and 0.05% ethylenediaminetetraacetic acid in 5% aqueous methanol for 2 min at 22,000 rpm (Ultraturrax, IKA, Germany). Two mg of D-isoascorbic acid were added as an internal standard. The homogenate was centrifuged at 10,000 rpm for 5 min at 4 ºC. Next 1.5 mL of supernatant were reacted with 0.5 mL of 1,2-phenylenediamine (3.33 mg/mL) diluted in methanol:water (5:95, v/v). The mix was kept for 37 min in the dark at room temperature. Afterwards, samples were passed through a 0.45-µm membrane filter and analysed by high pressure liquid chromatography (HPLC). The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), aquaternary pump (Model L-2130), a column oven (Model L-2300) and a diode array detector (Model L-2450). A reversed-phase C18 LiChroCart® column (250 x 4 mm, 5 µm-particle, Merck, Darmstadt, Germany), preceded by a precolumn (4 x 4 mm), was used. The injection volume was 40 µL and the oven temperature was 4 ºC. The mobile phase was a solution of methanol:water (5:95, v/v) that contained 5 mM hexadecyltrimethylammonium bromide and 50 mM ammonium dihydrogen phosphate, which was adjusted to pH 4.6. The flow rate was set at 1 mL min^{-1}. Ascorbic acid and D-isoascorbic acid were detected at 261 nm, whereas L-dehydroascorbic acid was detected at 348 nm. TVC was expressed as mg of ascorbic acid per 100 g of sample (fresh weight, FW).

Free radical scavenging activity was determined by the method of Brand-Williams et al. (1995) using $DPPH^{\bullet}$ as the free radical. Extraction was done as described by Chen et al. (2008) with some modifications. Two grams of persimmon pulp were mixed with 30 mL of 80% methanol. The solution was homogenised at 20,000 rpm for 2 min, followed by boiling in a water bath for 20 min to inactivate the PPO enzyme. The homogenate was immersed in an ultrasonic machine at room temperature for 15 min and centrifuged at 10,000 rpm for 20 min at 5 ºC. The resultant supernatant was then filtered and used as the persimmon extract. A second pulp extraction

was required to complete the extraction. The mix of both extracts was used for the analysis. Five methanolic dilutions from the supernatant were needed to relate the decrease in $DPPH^{\bullet}$ absorbance with sample concentration. Seventy five µL of extract were mixed with 225 µL of $DPPH^{\bullet}$ (24 ppm) and the mixture was kept in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 520 nm using a multiplate spectrum (Multiskan Spectrum, Thermo Fisher Scientific, Finland). $DPPH^{\bullet}$ radical scavenging activity was expressed as the effective concentration (EC_{50}). This value expresses the amount of persimmon extract needed to lower the initial $DPPH^{\bullet}$ concentration by 50%. Thus lower EC_{50} values mean greater antiradical capacity. Radical scavenging activity was expressed as g of persimmon fruit per kg of $DPPH^{\bullet}$.

TPC was measured following the method described by Chen et al. (2008). One gram of frozen sample was mixed with 15 mL of methanol with 1% hydrochloric acid. The mix was homogenised at 10,000 rpm for 1 min, immersed in an ultrasonic bath for 30 min, and centrifuged at 12,000 rpm for 20 min at 4 ºC. The supernatant was filtered and collected. Extraction was repeated and supernatants were combined for the analysis. Two methanolic dilutions were prepared with the extracts. Then 300 µL of the supernatant were mixed with 600 µL of Folin Ciocalteu reagent and 2.4 mL of sodium carbonate solution (200 mg/mL), and in this order. The mixture was incubated for 1 h in the dark at room temperature. The absorbance of the resulting solution was measured at 765 nm using a spectrum multiplate reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The results were expressed as mg of acid gallic for 100 g of persimmon fruit.

Carotenoids were determined as described by Wright and Kader (1997b). For the extraction, 5 g of sample were added to 10 mL of cold ethanol and homogenised for 3 min at 16,000 rpm. Eight mL of hexane were added and the sample was homogenised for another 2-minute period. The mixture was then centrifuged for 4 min at 5,000 rpm and 4 ºC, and the organic phase was transferred to a flask. Five mL of saturated sodium chloride were added to the contents of the centrifuge tube and stirred gently. Another 8 mL of hexane was added, and the mixture was homogenised for 1 min and centrifuged under the same conditions as described above. The resultant organic phase was then collected with the first extract. For saponification, 15 mL of 10% methanolic potassium hydroxide were added

to the extract. The flask was flushed with nitrogen, sealed and covered with aluminium foil to prevent oxygen and light. The flask was left at room temperature for 16 h with gentle shaking. Next the mixture was transferred to a separatory funnel to remove potassium hydroxide with 15 mL of 10% sodium chloride, followed by deionised water until the mixture had a neutral pH. The potassium hydroxide from the vial was extracted with another 10 mL of hexane. Both hexane extracts were evaporated under nitrogen until dryness and kept at -80 ºC until analysed. At the time of the analysis, samples were redissolved in 200 µL of methylene chloride and 1.8 mL of the mobile phase. The resuspended sample (1.5 mL) was filtered through a 0.45-µm nylon filter into amber vials and analysed by HPLC. The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), a quaternary pump (Model L-2130), a column oven (Model L-2300) and a diode array detector (Model L-2450). A reversed-phase C30 YMC-Pack column (250 x 4.6 mm, 5 µm-particle, Merck, Darmstadt, Germany) was used. The injection volume was 60 µL and the oven temperature was 4 ºC. The mobile phase consisted of acetonitrile, methanol that contained 0.05 M ammonium acetate, and methylene chloride 75:20:5 (v/v/v) that contained 0.1% butylated hydroxytoluene and 0.05% triethylamine. The flow rate was 1.5 mL/min. Detection was done at 450 nm. Identification of peaks was confirmed using standards of major carotenoids. The retinol equivalent (RE) was calculated on the basis of 1 RE = 6 µg of β-carotene or 12 µg of other provitamin A carotenoids (β-cryptoxanthin and α-carotene). The total carotenoid concentration was also quantified in a multiplate spectrum reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The resuspended sample (0.5 ml) was mixed with 2.5 ml of the mobile phase and measured within a wavelength range from 300 to 500 nm. The results were expressed as µg of total carotenoids per g of persimmon.

2.8. Statistical analysis

Statistical analyses were performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences among treatments were determined by least significant differences (LSD) when the analysis of variance (ANOVA) showed a significant *F*-value. Significant differences were defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

3.1. Colour change

Figure 1 shows the effect of the coatings and the antioxidant solution on the *L** and *a** values of the fresh-cut 'Rojo Brillante' persimmons processed in two MSs. The increased enzymatic browning of the fresh-cut persimmons during storage at 5 ºC was accompanied by an increase in *a** and a decrease in lightness (*L**). In both MSs, the coatings and the aqueous antioxidant solution significantly reduced enzymatic browning compared to the control samples. However, the differences in *L** and *a** between the control (water dip) and the other treatments (coatings and antioxidant aqueous solution) were larger in the fruits harvested earlier in the season (MS1) than in those harvested at the end of the season (MS2). In the fruits processed at MS2, no significant differences were observed in the *L** values between the control and treated samples, and *a** was the colour parameter that best described the effectiveness of the coatings and the antioxidant aqueous solution for controlling browning. In general, no significant difference between the coated and antioxidant-dipped persimmon slices was found, which indicated that the effect of reducing the enzymatic browning of persimmon slices was due to the presence of the antioxidants in the different edible coating formulations.

The effect of edible coatings to reduce the enzymatic browning of fresh-cut fruits has been reported by many authors. However in most of their works, antioxidant activity has been given by adding antibrowning agents, and very little is known about the contribution of coatings with no active ingredients to enzymatic browning (Rojas-Graü et al., 2009). Olivas et al. (2003) concluded that there was no effect from methylcellulose and methylcellulose-stearic acid coatings on controlling the enzymatic browning of fresh-cut 'Anjou' pears, and that the ascorbic acid present in the coating formulation was the principal factor responsible for delaying browning. However, Pérez-Gago et al. (2005a) showed that the edible coating matrix had an effect on the enzymatic browning of fresh-cut apples, and that whey protein-based coatings, with added antioxidants, proved more effective for controlling the enzymatic browning of fresh-cut apples than the HPMC-based coatings. This was attributed to a possible antioxidant effect of amino acid cysteine, which is present at high levels in whey protein. In a later work, the incorporation of ascorbic acid or cysteine into whey protein

concentrate-based coatings further retarded the enzymatic browning of fresh-cut apples, and proved more effective than antioxidants alone (Pérez-Gago et al., 2006). Yet in fresh-cut persimmons, the beneficial effect of such a combination depended on the concentration of the ascorbic acid incorporated into the whey protein concentrate-based coating formulation (Pérez-Gago et al., 2005b).

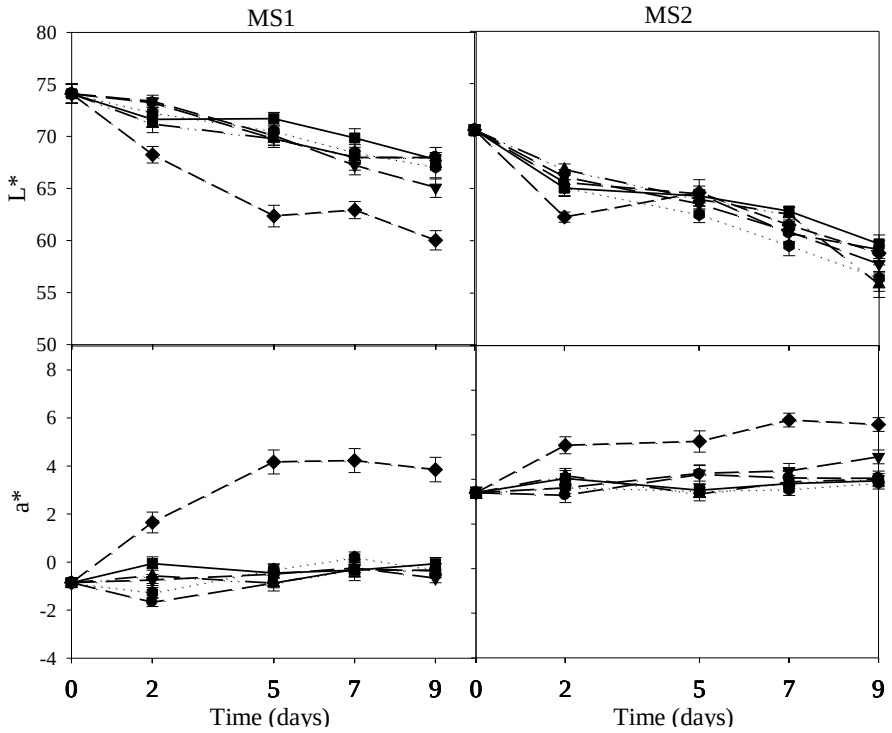

Fig. 1. Colour *L** and *a** values of fresh-cut 'Rojo Brillante' persimmon coated with SPI-coating (—■—), WPI-coating (—▲···), HPMC-coating (—●—), and pectin-coating (···●···), or dipped in antioxidant solution (10 g kg^{-1} citric acid + 10 g kg^{-1} $CaCl_2$) (—▽··) or water as control (—◆—). Persimmons were processed in two maturity stages (MS1 and MS2). SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose. Vertical bars show standard error.

3.2. Fruit firmness

Fruit firmness significantly decreased after processing and storage at 5 ºC for both the MSs (Fig. 2). Thus the firmness of the control persimmon slices decreased from 61.6±3.1 N on day 0 to 36.8±0.9 N on day 9 of storage in the fruits from MS1, and from 46.7±1.8 N to 24.7±1.4 N in the fruits from MS2. Coatings had little or no effect on maintaining the firmness of fresh-cut persimmons. Only in the processed persimmon fruits from MS1 did the samples coated with SPI and HPMC present significantly higher firmness values after 9 days of processing than the rest, although no differences were observed for the other storage days. The effectiveness of edible coatings on preventing firmness depends on many factors, such as coating composition, commodity or MS. Shon and Haque (2007) reported no effect of calcium caseinate, SPI and sour whey-based coatings on the firmness of fresh-cut apples, potatoes, carrots or onions. However, Tapia et al. (2008) reported higher firmness in fresh-cut papaya fruits coated with gellan or alginate than uncoated samples. Addition of ascorbic acid to a gellan-based coating doubled firmness compared with uncoated ones. Lee et al. (2003) reported that applying whey protein concentrate-based coatings to minimally processed apples reduced losses of firmness compared to uncoated samples, and that adding $CaCl_2$ to the coating formulation improved the maintenance of apple firmness during storage. On the contrary, a carrageenan-based coatings did not improve apple firmness and the addition of CA induced texture softening. In previous studies conducted with fresh-cut 'Rojo Brillante' persimmons, the application of acidic solutions like CA was effective for preventing enzymatic browning, but led to major tissue softening compared to untreated samples (Sanchís et al., 2015). Further studies have shown that the combination of this antioxidant with 10 g kg^{-1} $CaCl_2$ compensated the loss of firmness produced by the antioxidant, and maintained the firmness of the persimmon slices within the same range as the control samples (unpublished data).

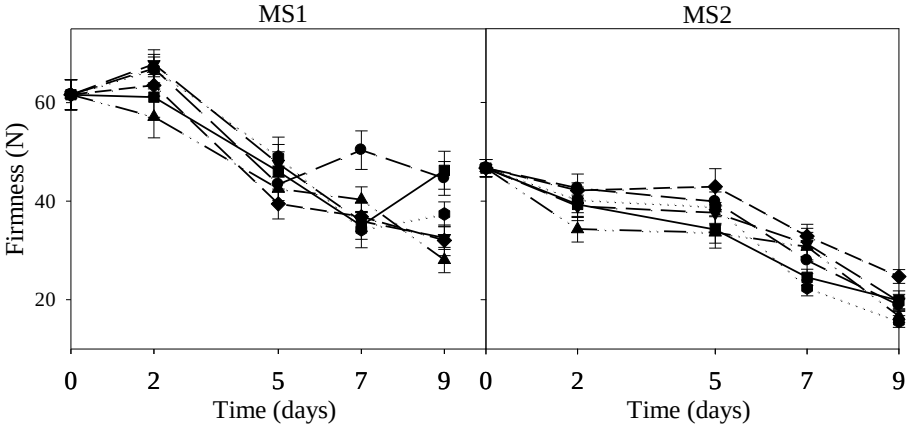

Fig. 2. Firmness of fresh-cut persimmons coated with SPI-coating (—■—), WPI-coating (—▲···), HPMC-coating (—●—), and pectin-coating (···⬡···), or dipped in antioxidant solution (10 g kg^{-1} citric acid + 10 g kg^{-1} $CaCl_2$) (—▽··) or water as control (—◆—). Persimmons were processed in two maturity stages (MS1 and MS2). SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose. Vertical bars show standard error.

3.3. Sensory quality

Figure 3 shows the visual quality of persimmon slices based on colour and general appearance, and a photograph of the samples after 9 storage days at 5 ºC. Uncoated samples were evaluated below the limit of marketability after 1 storage day for both MSs. At MS1, the samples coated with the SPI-based coating and those dipped in the antioxidant solution reached the limit of marketability by 7 days of storage, whereas those coated with HPMC and pectin-based coatings reached this limit after 9 storage days. The WPI-coated samples only reached 5 commercial shelf life days. At MS2, only the persimmon slices coated with the HPMC- and pectin-based coatings and those dipped in the aqueous antioxidant solution fell within the limit of marketability for 7 storage days at 5 ºC, but none of the treatments reached 9 storage days. These results contrast with the colour L^* and a^* values, which showed significant differences between the control and treated samples, but not among coatings (Fig. 1). These differences can be attributed to the darkening of some tissue areas during storage, which

differed from that observed as enzymatic browning caused by the cutting process (see the photograph of persimmon slices on storage day 9). Several studies have described this tissue darkening as a flesh disorder in whole persimmons known as 'flesh browning' (Novillo et al., 2014). Even though the cause of this disorder remains unknown, it has been related to pre-harvest nutritional deficiencies, mechanical injury during the postharvest period and/or the post-application of high CO_2 atmospheres to eliminate astringency, and further mechanical damage on the packing line (Besada et al., 2010; Zavrtanik et al., 1999). This disorder herein negatively affected the visual acceptance of fruits and reduced the commercial shelf life of the persimmon slices for both MSs. However, the application of the HPMC- or pectin-based coatings seemed to reduce it and helped maintain the limit of marketability of the fresh-cut persimmons for 9 or 7 storage days when harvested at the beginning or the end of the season, respectively.

Table 1 shows the effect of the different coatings on overall flavour, off-flavour, and the firmness of the fresh-cut 'Rojo Brillante' persimmons after 7 days at 5 ºC. Upon processing, persimmons were evaluated to have a good overall flavour, high firmness and no off-flavours in both MSs. The overall flavour of the persimmon slices remained within the range of acceptability for 7 storage days at 5 ºC, which indicates that the coatings did not negatively affect sensory quality. This correlated with the absence or very slight presence of off-flavours in the samples, with no significant differences among treatments. The high firmness scores obtained at harvest were maintained during storage, and the processed persimmons at MS1 were evaluated as being slightly firmer (sensory scores close to 4 = firm) than those processed at MS2 (sensory scores close to 3 = neither firm nor soft). As reported by Romaguera et al. (2009), the firmness evaluation confirmed the texture analysis results (Fig. 2). In their work, the correlation between the sensory and instrumental firmness of persimmon fruits, according to the data collected from different cultivars and storage periods, showed that an instrumental firmness, determined as the maximum force to penetrate tissue, above 30 N corresponded to sensory scores of 4-5 (firm-very firm), values that fell within the 20-30N range corresponded to scores of 3 (neither firm nor soft), and values below 10 N indicated the limit at which persimmon fruits were scored by the sensory panel as being soft.

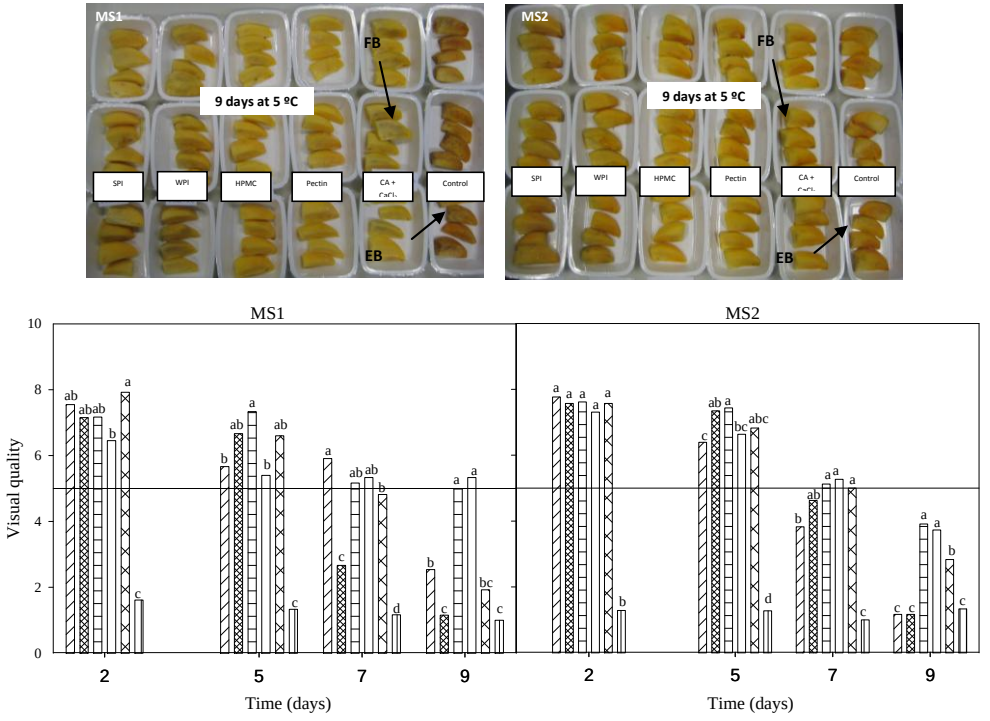

Fig. 3. Visual quality of the fresh-cut persimmons coated with SPI-coating (▨), WPI-coating (▩), HPMC-coating (▭), and pectin-coating (▭), or dipped in antioxidant solution (10 g kg^{-1} citric acid + 10 g kg^{-1} $CaCl_2$) (▨) or water as control (▥). Means within each storage time with the same letter are not different ($p<0.05$). Colour photographs show persimmon slices after 9 storage days at 5 ºC. Arrows show the differences between 'enzymatic browning' (EB) and 'flesh browning' (FB). Persimmons were processed in two maturity stages (MS1 and MS2). SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose.

Table 1. Flavour, off-flavour, and sensory firmness of coated and uncoated fresh-cut 'Rojo Brillante' persimmons processed in two maturity stages (MS1 and MS2) and stored at 5 ºC.

Storage (days)	Treatment	MS1			MS2		
		Flavour	Off-flavour	Firmness	Flavour	Off-flavour	Firmness
0		7.0 ± 1.2	1.0 ± 0.0	4.3 ± 0.5	7.0 ± 0.3	1.0 ± 0.0	4.3 ± 0.1
2	SPI-coating	6.1 ± 0.4ab	1.2 ± 0.1b	4.2 ± 0.26a	6.8 ± 0.3a	1.2 ± 0.1a	3.2 ± 0.2bc
	WPI-coating	6.2 ± 0.4ab	1.6 ± 0.1a	3.9 ± 0.2a	6.1 ± 0.5a	1.4 ± 0.2a	3.1 ± 0.1c
	HPMC-coating	6.0 ± 0.4ab	1.5 ± 0.2a	3.9 ± 0.3a	6.0 ± 0.5a	1.3 ± 0.1a	2.9 ± 0.2c
	Pectin-coating	5.3 ± 0.4b	1.6 ± 0.2a	4.1 ± 0.2a	5.9 ± 0.5a	1.6 ± 0.3a	3.7 ± 0.2ab
	Antioxidant dip	6.6 ± 0.3a	1.5 ± 0.3a	3.8 ± 0.3a	6.4 ± 0.3a	1.4 ± 0.2a	2.8 ± 0.2c
	Control	6.4 ± 0.3a	1.3 ± 0.1b	3.9 ± 0.2a	6.6 ± 0.4a	1.1 ± 0.1a	3.8 ± 0.1a
5	SPI-coating	7.0 ± 0.4a	1.1 ± 0.1a	4.0 ± 0.2a	6.1 ± 0.4b	1.2 ± 0.2a	2.8 ± 0.3c
	WPI-coating	6.3 ± 0.6ab	1.4 ± 0.3a	3.7 ± 0.4a	6.2 ± 0.5b	1.4 ± 0.3a	2.9 ± 0.2bc
	HPMC-coating	6.3 ± 0.5ab	1.7 ± 0.3a	4.1 ± 0.3a	6.0 ± 0.5b	1.4 ± 0.3a	3.4 ± 0.2ab
	Pectin-coating	5.8 ± 0.5b	1.7 ± 0.3a	4.4 ± 0.2a	6.1 ± 0.4b	1.5 ± 0.2a	3.7 ± 0.1a
	Antioxidant dip	5.9 ± 0.6b	1.9 ± 0.8a	3.2 ± 0.3a	6.2 ± 0.4b	1.5 ± 0.2a	3.7 ± 0.1a
	Control	5.9 ± 0.6b	1.1 ± 0.1a	3.9 ± 0.3a	6.9 ± 0.3a	1.1 ± 0.1a	3.6 ± 0.2a
7	SPI-coating	6.0 ± 0.4ab	1.1 ± 0.1a	3.5 ± 0.2a	5.8 ± 0.4ab	1.6 ± 0.2ab	3.0 ± 0.2ab
	WPI-coating	6.8 ± 0.2a	1.5 ± 0.3a	3.2 ± 0.2a	6.0 ± 0.4ab	1.5 ± 0.2ab	2.6 ± 0.1b
	HPMC-coating	5.7 ± 0.5bc	1.4 ± 0.2a	3.7 ± 0.3a	6.1 ± 0.4ab	1.5 ± 0.2ab	2.9 ± 0.2ab
	Pectin-coatin	5.2 ± 0.3c	1.6 ± 0.1a	3.5 ± 0.2a	5.4 ± 0.4b	1.9 ± 0.3a	2.8 ± 0.2b
	Antioxidant dip	6.5 ± 0.3ab	1.3 ± 0.2a	3.6 ± 0.2a	6.6 ± 0.4a	1.4 ± 0.2ab	2.9 ± 0.2ab
	Control	6.1 ± 0.4ab	1.3 ± 0.1a	3.6 ± 0.2a	6.7 ± 0.3a	1.3 ± 0.1b	3.4 ± 0.2a

SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose; Antioxidant dip = 10 g kg^{-1} citric acid + 10 g kg^{-1} CaCl2; Control = water.
Values are mean ± standard deviation (n=15)
The values within storage time followed by the same letter indicate that mean values were not significantly different by the ANOVA test ($p \leq 0.05$).

3.5. Bioactive compounds

There are many factors that affect the nutritional values of fresh-cut fruits. Table 2 shows the ANOVA F-ratios for the effect of the main factors and their interactions on the TVC, radical scavenging activity, TPC and total carotenoid content of the fresh-cut 'Rojo Brillantes' stored at 5 ºC. MS had a significant effect on TVC, TPC and radical scavenging activity, whereas coating treatment had an effect only on TVC and total carotenoid content. Post-processing storage time affected only TVC. In general, the fruits processed at MS1 obtained lower TVC and TPC content values, but higher radical scavenging activity values (Table 3, Table 4). Similar trends have been reported in previous works done with fresh-cut persimmons (Sanchís et al., 2015). Although the effect of coating application and storage time at 5 ºC on TVC was significant, it had no clear effect, and the observed differences could be attributed to biological variation. Other authors like Gil et al. (2006) have reported an increase in TVC during storage at 5 ºC over 9 days in pineapple pieces and strawberry slices, and a decrease in fresh-cut mangoes, cantaloupes, watermelons and kiwis. In fresh-cut 'Fuyu' persimmons, Wright and Kader (1997a) reported a loss in TVC on day 1 after cutting, but values then recovered to levels that did not significantly differ from day 1. These variations were attributed to biological variations between fruits. Similarly, the literature reports some differences in the effect of edible coatings on vitamin C and other bioactive compounds of fresh-cut fruits. The application of gellan, alginate or pectin edible coatings that contained N-acetylcysteine and glutathione did not seem to increase the TVC of fresh-cut pears, but helped reduce vitamin C losses and maintain antioxidant capacity during storage (Oms-Oliu et al., 2008). The addition of ascorbic acid to alginate and gellan edible coatings increased TVC content in fresh-cut papaya fruits compared to either the coating with no antioxidant or the uncoated control (Tapia et al., 2008). Likewise, an alginate-based edible coating amended with ascorbic as antioxidant increased the TVC and total antioxidant capacity of fresh-cut mangoes compared to the coating with no antioxidant or the uncoated control (Robles-Sánchez et al., 2013). However, a multilayered edible coating based on sodium alginate, pectin and calcium chloride, with a microencapsulated antimicrobial complex (beta-cyclodextrin and trans-

cinnamaldehyde), did not help retain the vitamin C content of fresh-cut pineapples (Mantilla et al., 2013).

The total carotenoid content of minimally processed 'Rojo Brillante' persimmons ranged from 100 to 800 (μg/100g) for both MSs, and showed wide variability that did not seem to depend on MS or storage time at 5 ºC after processing (Table 2). These values fell within the same range as those obtained in a previous work conducted with 'Rojo Brillante' persimmons (Sanchís et al., 2015). It was reported a significant effect of MS on total carotenoid content, which was not the case in the present work. Individual carotenoids were also analysed, and the results are shown in Fig. 4. Eight individual carotenoids were found, of which β-cryptoxanthin and β-carotene were the most abundant. The concentrations of these two major carotenoids were unaffected by MS, storage time at 5 ºC or the application of different edible coatings. The RE provided by the two major provitamin A carotenoids (β-carotene and β-cryptoxanthin) of 'Rojo Brillante' persimmons ranged between 10 and 24 μg/100 g of FW, which is between 1.25-3% of the recommended female daily allowance in a 100 g serving. These values were lower than those reported for other persimmon cultivars (Wright and Kader, 1997b; Giordani et al., 2011; Plaza et al., 2012).

Table 2. Analysis of covariance for the effect of coating treatment, maturity stage (MS) at harvest, storage (S) time of fresh-cut persimmons at 5 ºC, and their interactions on the total vitamin C content, free radical scavenging activity, total phenolic content and total carotenoid content of fresh-cut 'Rojo Brillante' persimmons.

	Vitamin C	Free radical scavenging activity	Total phenolic content	Total carotenoids
Treatment	2.44*	0.91	0.94	2.57*
MS	6.42*	16.15*	536.52*	2.45
S	87.76*	2.10	0.30	2.75
Interactions				
Treatment x MS	13.9*	13.82*	1.27	2.59*
Treatment x S	6.11*	4.98*	3.19*	9.89*
MS x S	15.68*	39.26*	8.65*	14.10*
Treatment x MS x storage	3.98*	3.46*	1.60	4.49*

Numerical values are the F-ratio of the variance.
* Significant F-ratios at $p \leq 0.05$

Table 3. Total vitamin C and free radical scavenging activity of coated and uncoated fresh-cut 'Rojo Brillante' persimmons processed in two maturity stages (MS1 and MS2) and stored at 5 ºC.

Storage (days)	Treatment	Total vitamin C (mgAA/100g)		Free radical scavenging activity (g /kg DPPH•)	
		MS1	MS2	MS1	MS2
0		191.8 ± 29.0B	354.6 ± 40.4A	253.9 ± 18.3A	235.4 ± 8.67A
2	SPI-coating	467.2 ± 17.7aA	197.7 ± 17.5bcB	246.2 ± 13.3bA	297.2 ± 38.7cA
	WPI-coating	347.7 ± 17.4bA	227.6 ± 10.6abB	258.2 ± 26.9abB	479.5 ± 34.2aA
	HPMC-coating	172.7 ± 11.3deB	235.5 ± 11.8aA	278.1 ± 17.8abA	333.5 ± 23.9bcA
	Pectin-coating	150.7 ± 9.6eA	160.5 ± 5.7cdA	276.4 ± 23.4abB	391.0 ± 27.2bA
	Antioxidant dip	256.7 ± 23.9cA	157.3 ± 14.5dB	312.5 ± 10.5aA	381.6 ± 30.7bcA
	Control	238.8 ± 40.0cdA	192.0 ± 11.1bcdA	300.9 ± 17.2abA	308.0 ± 31.5bcA
5	SPI-coating	112.2 ± 6.5bB	224.3 ± 28.8aA	273.2 ± 26.0bB	450.2 ± 31.1bA
	WPI-coating	106.4 ± 16.8bA	161.3 ± 17.3aA	179.7 ± 26.0cB	399.6 ± 29.0bcA
	HPMC-coating	92.9 ± 10.5bB	212.4 ± 30.2aA	356.9 ± 31.0aA	377.6 ± 17.6bcdA
	Pectin-coating	113.7 ± 9.5bB	196.9 ± 23.2aA	234.7 ± 21.0bcB	592.7 ± 27.5aA
	Antioxidant dip	112.0 ± 12.4bB	174.7 ± 10.6aA	247.7 ± 9.5bcB	317.0 ± 11.5cdA
	Control	229.0 ± 46.3aA	219.0 ± 20.0aA	294.1 ± 32.6abA	295.4 ± 38.3dA
9	SPI-coating	243.3 ± 34.1aA	2999 ± 40.4abA	411.6 ± 32.0abA	408.8 ± 37.9aA
	WPI-coating	148.1 ± 18.6bcB	371.1 ± 32.1aA	384.1 ± 16.6abA	317.3 ± 13.3bB
	HPMC-coating	205.4 ± 22.6abA	190.3 ± 41.1bA	324.5 ± 21.6bA	289.6 ± 18.4bA
	Pectin-coating	179.6 ± 23.8abcB	367.9 ± 56.6aA	354.8 ± 36.6bA	367.8 ± 26.4abA
	Antioxidant dip	171.2 ± 42.3abcA	273.5 ± 92.7abA	377.8 ± 40.3abA	352.1 ± 14.1abA
	Control	130.4 ± 16.4cB	398.1 ± 24.3aA	452.6 ± 43.2aA	207.2 ± 30.9cB

SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose; Antioxidant dip = 10 g kg^{-1} citric acid + 10 g kg^{-1} CaCl2; Control = water.
Values are mean ± standard error
For each MS and storage time, small letters indicate significant differences among treatments.
For each treatment, capital letters indicate significant differences between MS.

Table 4. Total phenolic content and total carotenoids of coated and uncoated fresh-cut 'Rojo Brillante' persimmons processed in two maturity stages (MS1 and MS2) and stored at 5 ºC.

Storage (days)	Treatment	Total phenolic content (mg GA/100g)		Total carotenoid content (µg /100g)	
		MS1	MS2	MS1	MS2
0		9.0 ± 0.6A	6.0 ± 0.8B	251.7 ± 16.9A	326.4 ± 26.6A
2	SPI-coating	10.1 ± 1.1aB	15.3 ± 0.5aA	435.0 ± 69.4bcA	482.6 ± 34.1aA
	WPI-coating	8.5 ± 0.8abB	12.4 ± 0.4bcA	788.3 ± 39.3aA	282.7 ± 23.1bcB
	HPMC-coating	8.3 ± 0.7abB	13.4 ± 0.7bcA	832.9 ± 61.3aA	358.8 ± 35.9abcB
	Pectin-coating	8.8 ± 0.5abB	14.6 ± 1.7abA	302.0 ± 13.7cA	91.4 ± 15.8dB
	Antioxidant dip	7.8 ± 0.3bcB	14.0 ± 0.4abA	533.0 ± 65.1bA	203.6 ± 55.5cdA
	Control	5.7 ± 0.5cB	11.6 ± 0.7cA	460.4 ± 18.8bcA	430.4 ± 79.9abA
5	SPI-coating	6.8 ± 0.6aB	13.1 ± 0.5bA	275.6 ± 87.4cdA	324.6 ± 71.4cA
	WPI-coating	6.6 ± 0.4abB	16.8 ± 0.9aA	774.2 ± 7.6aA	528.4 ± 26.9bA
	HPMC-coating	6.2 ± 0.3aB	14.3 ± 0.5bA	444.8 ± 8.5bcA	497.4 ± 50.5bA
	Pectin-coating	7.0 ± 0.4aB	15.4 ± 0.5abA	194.4 ± 51.3dA	464.9 ± 56.6bA
	Antioxidant dip	5.5 ± 0.4aB	14.0 ± 0.5bA	792.4 ± 36.4aA	747.9 ± 0.1aA
	Control	6.3 ± 0.3aB	14.5 ± 0.5abA	620.2 ± 70.7abA	480.0 ± 32.4bA
9	SPI-coating	5.7 ± 0.4bB	14.8 ± 0.4aA	199.9 ± 33.0cB	391.9 ± 14.6bcA
	WPI-coating	5.9 ± 0.2bB	13.5 ± 0.5aA	225.1 ± 6.6bcA	615.9 ± 6.8aA
	HPMC-coating	6.2 ± 0.4bB	16.7 ± 0.5aA	467.5 ± 72.9aA	484.7 ± 19.3abA
	Pectin-coating	6.0 ± 0.2bB	17.3 ± 0.8aA	425.7 ± 9.6abA	555.4 ± 28.3abA
	Antioxidant dip	7.9 ± 0.2aB	14.5 ± 0.3aA	303.0 ± 50.9bcA	244.7 ± 25.9cA
	Control	8.5 ± 0.6aB	15.8 ± 0.8aA	235.1 ± 18.5bcA	228.4 ± 19.8cA

SPI = soy protein isolate; WPI = whey protein isolate; HPMC = hydroxylpropyl methylcellulose; Antioxidant dip = 10 g kg^{-1} citric acid + 10 g kg^{-1} CaCl2; Control = water.
Values are mean ± standard error
For each MS and storage time, small letters indicate significant differences among treatments.
For each treatment, capital letters indicate significant differences between MS.

CONCLUSION

All the tested edible coatings and the antioxidant treatment proved effective for controlling enzymatic browning in fresh-cut persimmons. However, the limit of marketability was conditioned by a burst of a disorder known as 'flesh browning', which negatively affected the visual quality of the samples. The application of the HPMC- or pectin-based coatings helped extend the commercial shelf life of persimmon fruits to 9 days at 5 ºC. Nutritional and flavour quality were not negatively affected by cutting, coating treatments or storage at 5 ºC. Future studies are recommended to improve the functionality of antioxidant edibles coatings as vehicles for antimicrobial agents.

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Chapter 7

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 moulds 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 moulds, 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.

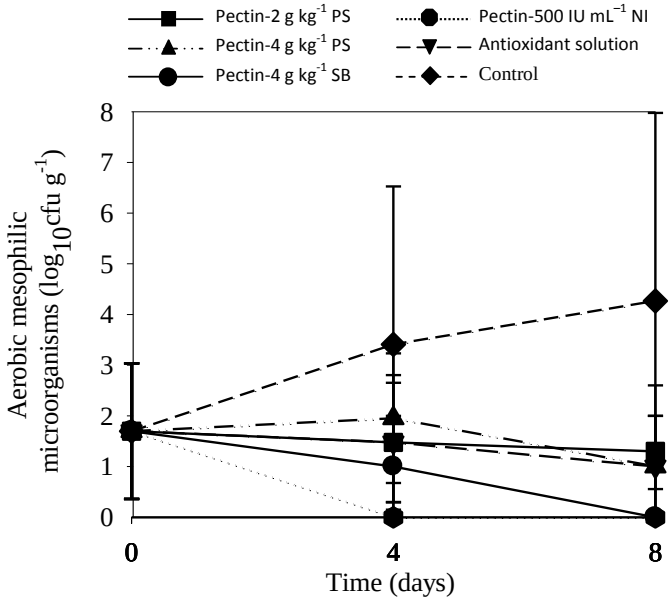

Fig. 1 Growth of aerobic mesophilic bacteria on fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored for 8 days at 5 ºC. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid;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

'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.

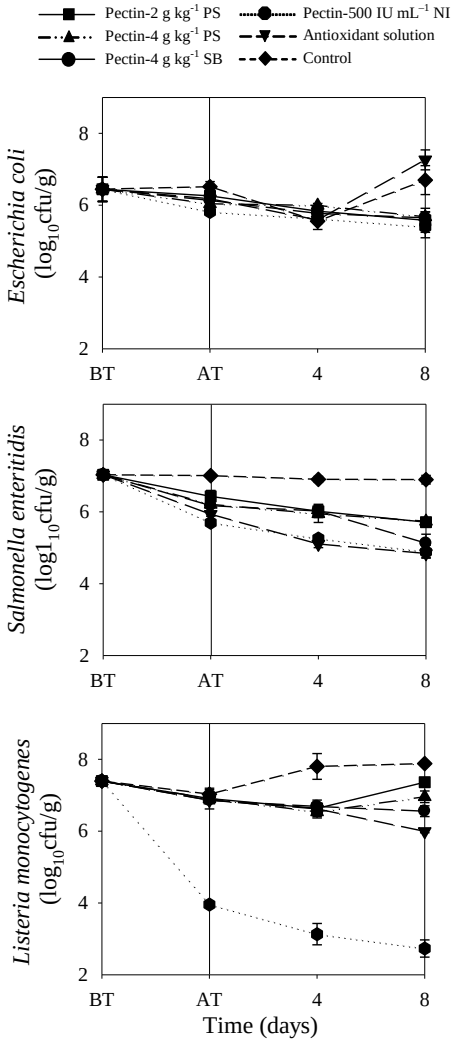

Fig. 2 Populations of *Escherichia coli* (A), *Salmonella enteritidis* (B) and *Listeria monocytogenes* (C) on artificially inoculated fresh-cut 'Rojo Brillante' persimmon plugs before (BT) and after (AT) dipping in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based 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.

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** and hue values (Fig. 3). The lowest hue and the highest *a** values 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 whey protein-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 antibrowning agents. The mechanism of enzymatic inhibition differs vastly for each type of antibrowning 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.

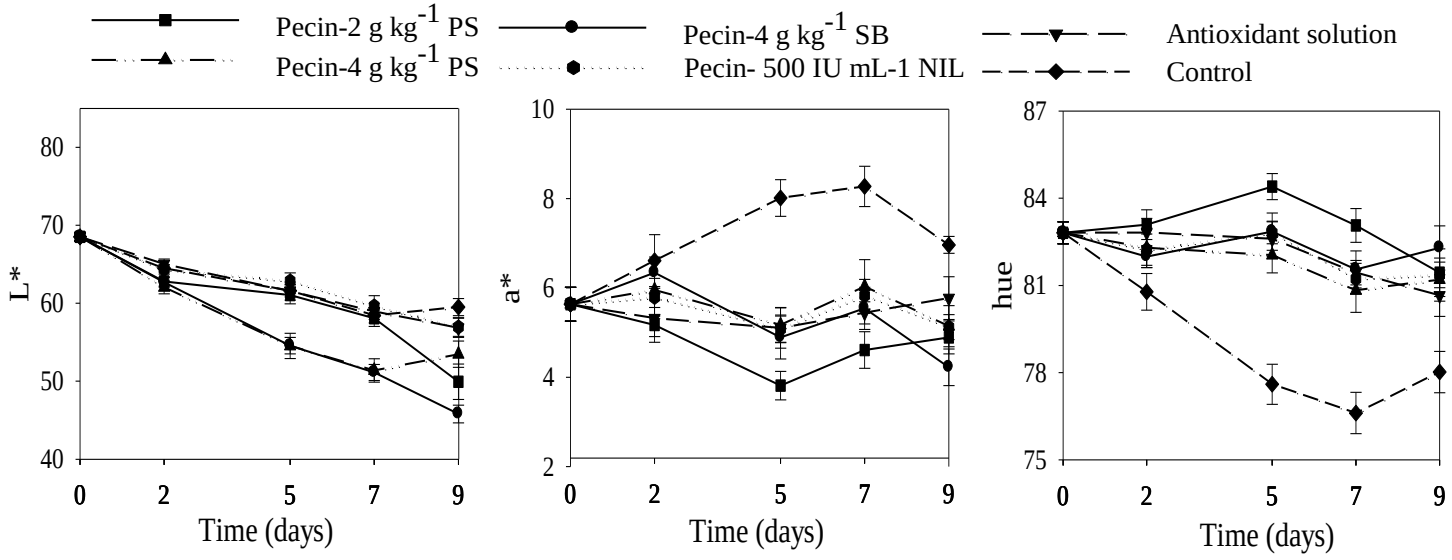

Fig. 3 Flesh color of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride.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

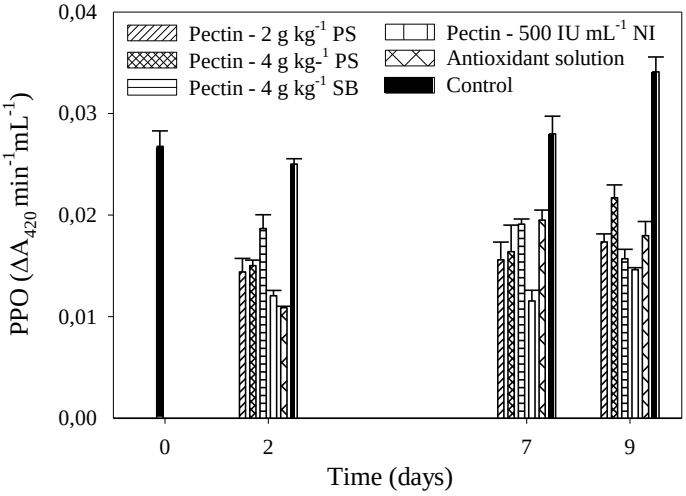

Fig. 4 Polyphenol oxidase (PPO) activity in fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: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

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.

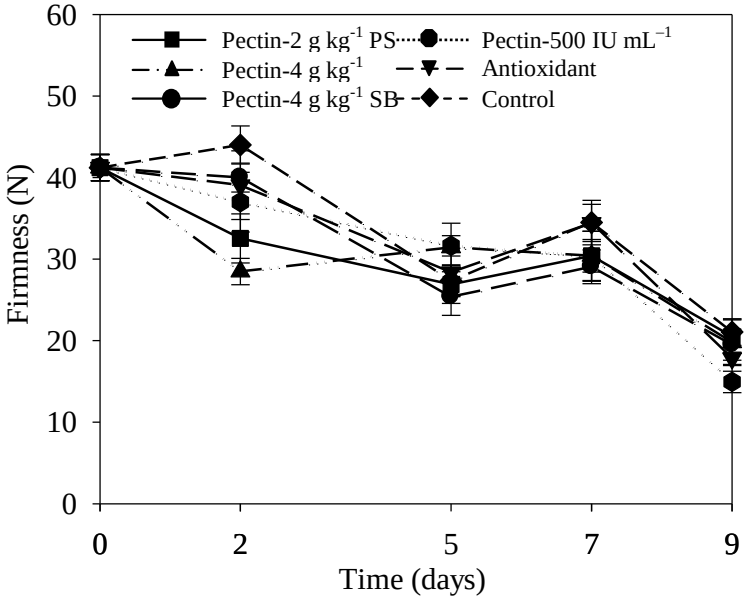

Fig. 5 Firmness of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. Vertical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

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.

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.

	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

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.

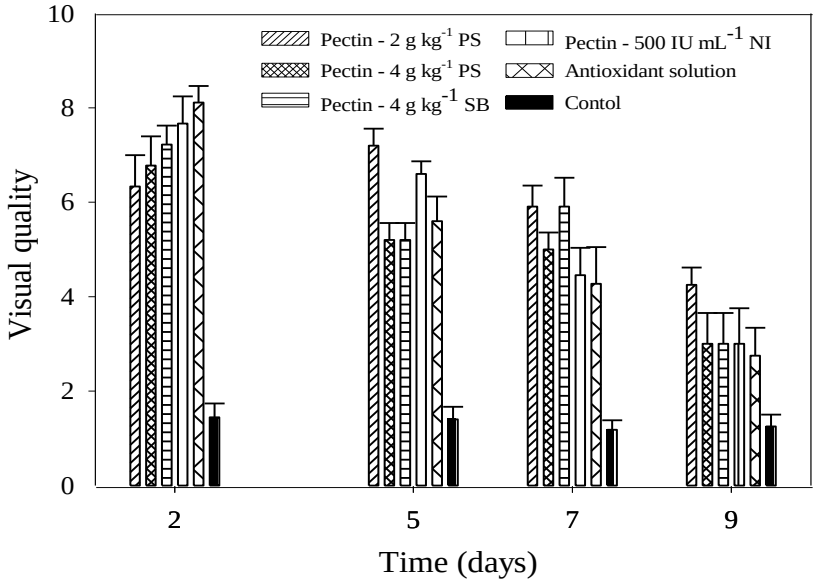

Fig. 6 Visual quality of fresh-cut 'Rojo Brillante' persimmons dipped in water (Control), antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or pectin-based coatings and stored at 5 ºC for 9 days. PS: potassium sorbate; SB: sodium sorbate; NI: nisin; CA: citric acid; $CaCl_2$: calcium chloride. 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.

4. CONCLUSION

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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Chapter 8

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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Abstract

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 antioxidant 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. 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*. Overall, 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, antioxidants, antimicrobial, shelf-life.

INTRODUCION

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 (Sanchís et al., 2015a). Furthermore, cut surfaces can provide both attachment opportunities and entry points for microorganisms (O'Beirne et al., 2014). 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 (Alegre et al., 2010).

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 (Forney, 2007). 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 (Kader and Ben-Yehosua, 2000). 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 antioxidant 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 (Pérez-Gago et al., 2005). 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. (Pérez-Gago et al., 2006; Oms-Oliu et al., 2008; Tapia et al., 2008). 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 antioxidant 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 (Sanchís et al., 2015b).

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 (Mastromatteo et al., 2011), and improved the microbiological quality of fresh-cut strawberries coated with chitosan (Campaniello et al., 2008). 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 (Ghidelli et al., 2010). 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 antioxidant 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.

MATERIAL AND METHODS

2.1. 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.

2. 2. 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 antioxidant 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 IU mL^{-1} (Coralim Aditivos S.L., Valencia, Spain). The coating emulsion was 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), *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}.

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 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, perforated 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 (four 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.

2.5. 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.

2.6. Microbiological growth in fresh cut persimmons

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.

2.7. 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 (Alegre et al., 2010). 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 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.

2.8. 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.

2.9. 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).

2.10. 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).

2.11. 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 (Gorny et al., 2002). 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.

2.12. Statistical analysis

The statistical analysis was performed with the software 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 significant *p*-value. Significant differences were defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

3.1. Headspace gas composition

Fig. 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 (Rojas-Graü et al., 2007; Raybaudi-Massilia et al., 2008) and in apple and mango slices coated with cassava starch (Fontes et al., 2008).

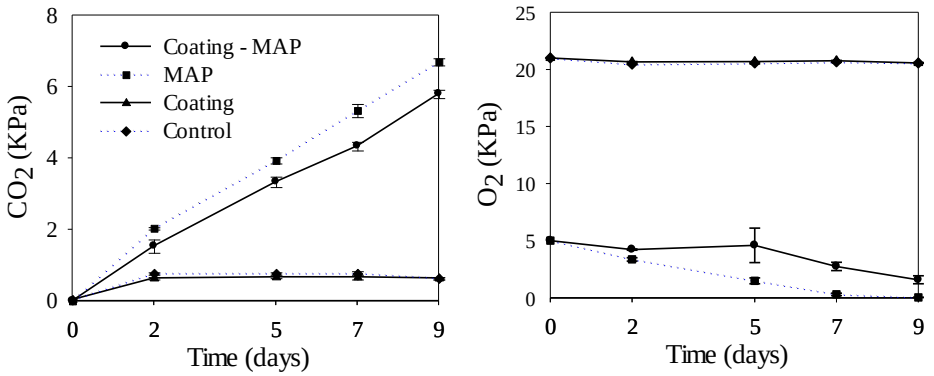

Fig. 1. Concentration of CO_2 and O_2 in the headspace gas composition of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Vertical bars represent the standard error.

3.2. 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). Fig. 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 (Simón et al., 2005; Oms-Oliu et al., 2008; Li et al., 2012) and reduced the development of aerobic psycrhrotrophic bacteria and *Pseudomonas* in leaf spinaches (Tudela

et al., 2013). 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. (2009) 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.

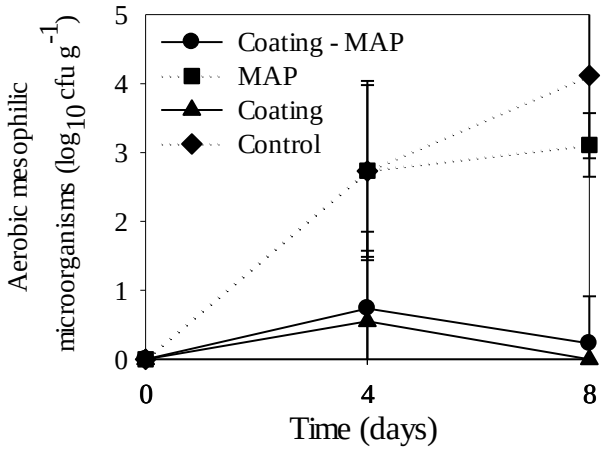

Fig. 2. Growth of aerobic mesophilic bacteria in uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or active modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 8 days at 5ºC. Vertical bars show the standard error.

In a previous work (Sanchís et al., 2015b), a similar pectin-based edible coating amended with 500 IU mL^{-1} NI totally inhibited aerobic mesophiles in fresh-cut 'Rojo Brillante' persimmon. NI has a broad activity spectrum against gram-positive bacteria, but do not significantly inhibit gram-negative bacteria, yeasts or moulds (Thomas and Delves-Broughton, 2005). 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 (Ross et al., 2003). In our coating formulation, CA and $CaCl_2$ were added as antioxidants 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 (Aguayo et al., 2008; Freitas et al., 2013).

3.3. 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 Fig. 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.

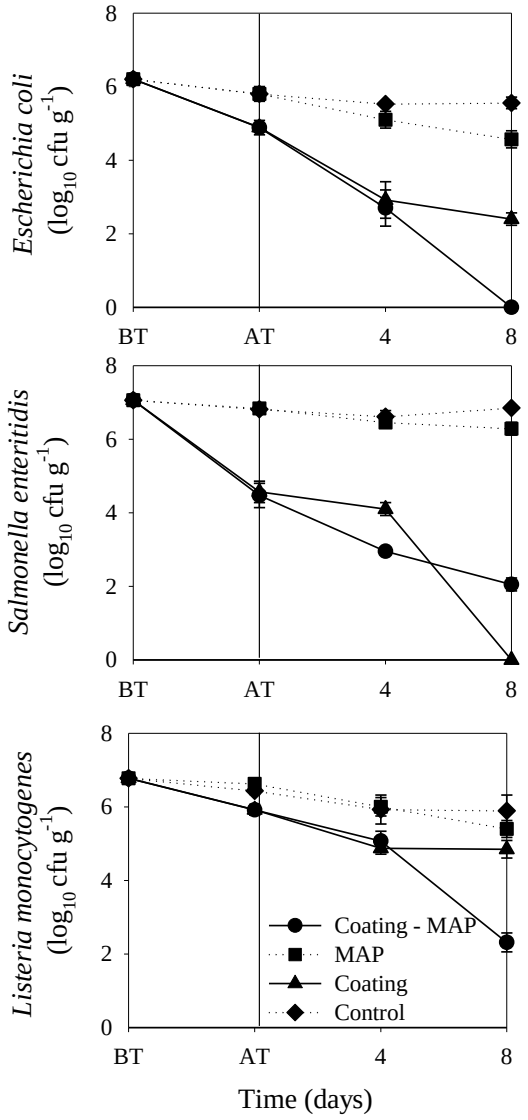

Fig. 3. Populations of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* in uncoated and pectin-based coated minimally processed 'Rojo Brillante' persimmon plugs before (BT) and after treatment (AT), and after 4 and 8 days of storage at 5 ºC in air or active modified atmosphere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

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 (Sanchís et al. 2015b). 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 inhabiting the growth of Gram-positive bacteria is well-known. For example, NI inhibited the growth of *L. monocytogenes* in processed mangoes or melons (Teixera-Barbosa et al. 2013; Ukuku and Fett, 2004). 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 (Davies and Adams, 1994; Mazzotta and Montville, 1997; Nawrocki et al., 2014). 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 (Thomas and Delves-Broughton, 2005). 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 (Stevens et al., 1992; Cutter and Siragusa, 1995). 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.

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

Fig. 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).

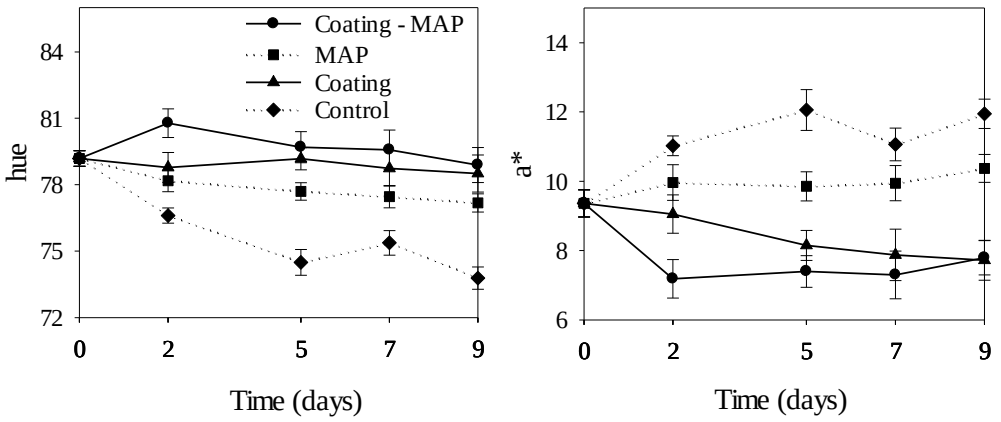

Fig. 4. Flesh color hue and *a** values of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Vertical bars show the standard error.

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 antioxidants (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 (Oms-Oliu et al., 2008) and the incorporation of ascorbic acid into an alginate-based coating contributed to colour retention of fresh-cut mango (Robles-Sánchez et al., 2013).

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 antioxidant agents and MAP has been evaluated by our group on fresh-cut artichoke, eggplant, and persimmon (Ghidelli et al., 2010, 2013, 2015). 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 (Ghidelli et al., 2014). 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 (Ghidelli et al., 2010).

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 (Ibrahim et al., 2004). In persimmon, the optimum PPO activity has been reported to fall within the pH range of 5.5-7.5, depending on the substrate (Núñez-Delicado et al., 2003; Özen et al., 2004). 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

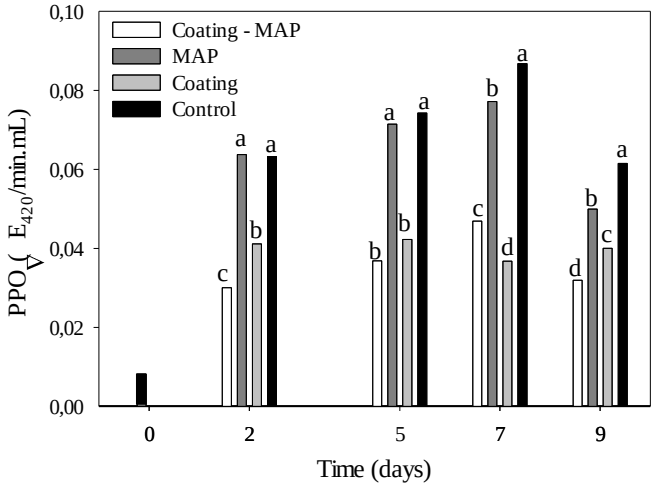

Fig. 5. Polyphenol oxidase (PPO) of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. For each storage time, bars with a different letter are significantly different at the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

3.5. 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 (Toivonen and Hampson al., 2009). For example, the application of 4 kPa O_2 + 5 kPa CO_2 delayed firmness loss in fresh-cut pineapple (Pan et al., 2015), and similar atmospheric conditions positively influenced firmness in fresh-cut apples compared to air conditions (Cortellino et al., 2015). In this work, the application of active MAP only helped to retain firmness of uncoated samples, whereas coated samples were not benefited by MAP. 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 (unpublished data)

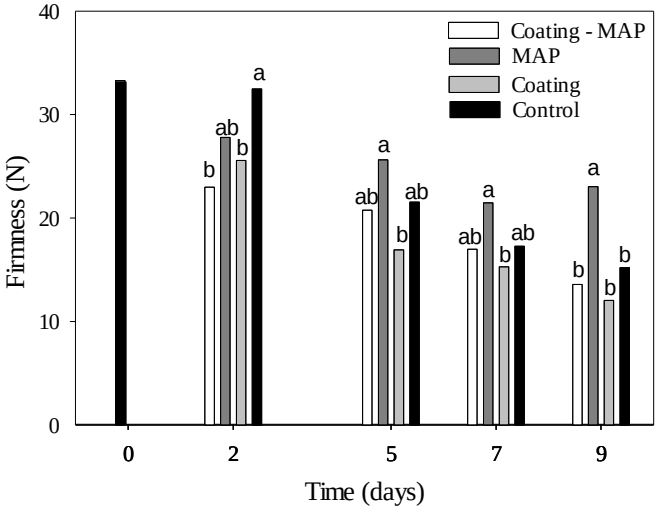

Fig. 6. Firmness of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmon packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. For each storage time, bars with a different letter are significantly different at the 95% level.

3.6. 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.

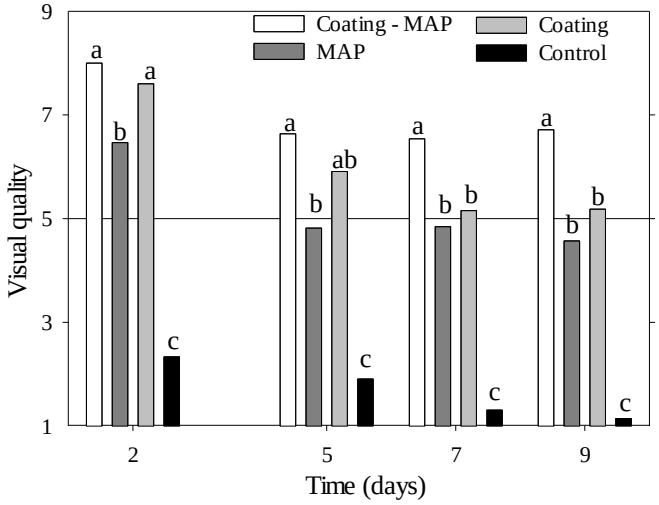

Fig. 7. Visual quality of uncoated and pectin-based coated fresh-cut 'Rojo Brillante' persimmons packed in air or modified atmosphere packaging (MAP; 5 kPa O_2, balance N_2) and stored for 9 days at 5 ºC. Visual scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair,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

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.

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

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.

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 was 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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GENERAL DISCUSSION

The objective of the present thesis was to develop optimum procedures for processing and marketing 'Rojo Brillante' persimmon into a fresh-cut product with the maximum shelf life and best physicochemical, nutritional, sensory and microbiological quality. A high-quality product starts with fruits of superior raw material quality. Preharvest factors such as genetic background, agronomic practices, environmental conditions, and stage of maturity at harvest, affect the quality of the fresh-cut commodity. Moreover, physical damage during processing results in surface browning, tissue softening, loss of flavour, and increased susceptibility to microbial spoilage and nutritional compounds degradation that significantly reduces the fruit´s shelf life. This Thesis has focused in the study of antioxidants, storage in controlled atmosphere and modified atmosphere packaging and edible coatings to extend the shelf-life of fresh-cut persimmon 'Rojo Brillante'. Furthermore, the effects of these technologies have been studied in fruits at different stages of maturity (MS).

1. Effect of degree of ripeness of persimmon 'Rojo Brillante' on processing

Maturity at harvest is the most important factor that determines storage shelf-life and final fruit quality. In most fruits, the maturity stage, as determined by harvest date and/or postharvest ripening, appears essential for maintaining the quality and for accomplishing an appropriate shelf life of fresh-cut produce.

The harvest season of 'Rojo Brillante' cultivar takes place between October and December. After the astringency has been removed, fruit firmness is the main property taken into account in order to rate quality. Nevertheless, the external colour (CI=1,000 *a/Lb*) of the persimmon is the property used as a non-destructive index for harvesting. In this Thesis, fruits were harvested either at the beginning of the season (early- to mid-October), which corresponded to MS1, or mid-end of the season (mid-November to early-December), which corresponded to MS2. For these harvest periods, the range of CI was -1.7–1.5 and 9–17 for MS1 and MS2, respectively. These corresponded to a fruit firmness at harvest of 45–69 N and 21–40 N for MS1 and MS2, respectively. From these

values, the lowest firmness corresponded to the highest CI of the fruits. In particular, it is important to remark the gradual decrease in the firmness of the pulp at harvest that went from values close to 70 N at the beginning of the season to values around 20 N at the end of the season; whereas fruits from mid-October to mid-November had an average firmness close to 40 N.

At harvest, the persimmon slices with MS1 had higher *L** and lower *a** values than those with MS2. This can related to an increase in carotenoid content as the MS advances, which is an important factor that influences changes in colour (Zhou et al., 2011). When studying the effect of the antioxidants (ascorbic acid (AA), citric acid (CA), cysteine (Cys) and calcium chloride ($CaCl_2$)) to control enzymatic browning in persimmon fruits, these were more effective for the persimmon fruits harvested at an earlier MS (MS1) than those harvested at more advanced maturity (MS2). Furthermore, in fruits harvest with MS2, the effectiveness of antioxidant treatments diminished as the time of storage of the whole fruit at 15 ºC was prolonged. These results were later confirmed when AA and CA were combined with $CaCl_2$ (i.e. colour differences between control and antioxidant treated persimmons were less marked in late season fruits). Similarly, the effect of the different controlled atmospheres and edible coatings tested in enzymatic browning was more marked in fruits with MS1 than fruits with MS2. These differences may be due to changes in the phenolic content and PPO activity of fresh-cut persimmons at different MSs. Similar behaviour was reported in fresh-cut apples, where enzymatic browning was more efficiently controlled by antioxidants in mature-green and partially-ripe fruit than in ripe tissue (Soliva-Fortuny et al., 2002; Rojas-Graü et al., 2007). This result was attributed to chloroplast disintegration as maturity increases, which would caused the solubilisation of PPO and an increase in browning oversensitivity.

On the other hand, the decrease in fruit firmness of processed fruit during storage at 5 ºC in fruits harvested at the end of the season was more important than in fruits harvested earlier in the season. In general, the fruits with firmness at harvest around 60 N displayed a firmness loss close to 15-20% after 9 storage days, whereas those with firmness at harvest around 40 N experienced firmness losses that could reach up to

45-50%. This drop in fruit firmness can condition the quality of fresh-cut slices when firmness at harvest is below 20 N, as it was observed in the most advanced mature persimmons studied in this Thesis (Chapter 1).

2. Browning control of fresh-cut 'Rojo Brillante' persimmon

Surface browning is probably the most common quality defect of fresh-cut fruit and the factor most limiting shelf-life. Common evaluation of enzymatic browning is based on reflectance measurements (*L**, *a**, *b*)* on the cut surface. However, it is also necessary to assess the visual appearance of persimmon slices, based on colour and general appearance, by a sensory panel with the aim of determining if the colour differences observed instrumentally could be also observed visually.

After processing, increased enzymatic browning of the fresh-cut persimmons during storage at 5 ºC was accompanied by an increase in *a** and a decrease in *L** values. In general, for all the technologies studied, the control samples (untreated) always had the lowest *L** values and highest *a** values and they were always evaluated as poor or inedible after 1-2 days of processing as a consequence of enzymatic browning. The application of antioxidants reduced enzymatic browning, being AA and CA the most effective ones. This result confirmed previous findings by Ghidelli et al. (2013) in fresh-cut 'Rojo Brillante' persimmons, who reported that AA and CA were the most effective treatments from a wide range of antioxidants to reduce enzymatic browning. This was attributed to a reduction in the pH below the optimum for persimmon PPO activity, which has been reported to fall within a pH range of 5.5-7.5 (Núñez-Delicado et al., 2003; Özen et al., 2004) and to the AA acting as reducing agent. Our work also showed a loss of effectiveness of CA in the fruits harvested late in the season (MS2, CI of 17.6±1.7 and 20.9±5.4 N) if compared to those harvested earlier (MS1, CI of 1.5±1.0 and 45.6±4.7 N), which was reflected in a lower commercial shelf life. Thus in fruits with MS2, AA maintained persimmon slices within the limit of marketability over a storage period lasting 6-8 days, whereas CA only achieved 4 days of commercial shelf life at 5 ºC.

In a later study, the combination of $CaCl_2$ with AA or CA controlled enzymatic browning in both MSs studied, and no differences were

observed among treatments for neither the *L** nor the *a** values, showing a synergic effect with such a combination. However, although all the antioxidant treatments tested proved effective to control enzymatic browning in fresh-cut persimmon, the limit of marketability was reached by day 7 for the samples processed with MS2, and this period was shorter for the samples with MS1. These results contrast with previous work which reported limits of marketability of persimmon slices to fall within the range of 6-8 days by 10 g L^{-1} AA or CA for persimmons with MS1. Furthermore, in the same work, CA lost effectiveness in the fruits harvested in the second half of the season. The differences between colour evaluation and visual quality of the persimmon processed at MS1 were due to a burst of the disorder known as 'flesh browning', which negatively affected the visual quality of the samples, and it was more severe in fruits with MS1 that with MS2. Overall, the combination 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ resulted the best antioxidant treatment to maintain the visual quality of fresh-cut persimmons for up to 7 days at 5 ºC.

The selection of optimum O_2 and CO_2 concentrations for modified atmosphere packaging (MAP) was preceded by a study of different controlled atmospheres. Overall, the combination of antioxidant dips and a controlled atmosphere composed of 5 kPa O_2 (balance N_2) proved to be the most effective combination to prevent enzymatic browning and also for visual quality, which reached the limit of marketability by storage day 9 at 5 ºC; whereas, the application of high CO_2 concentrations induced 'flesh browning' in some tissue areas. Even though the cause of this disorder remains unknown, it has been related to pre-harvest nutritional deficiencies, mechanical injury during the postharvest period and the post-application of high CO_2 atmospheres to eliminate astringency (Besada et al., 2010; Zavrtanik et al., 1999, Novillo et al., 2014a, 2014b). Thus, Novillo et al. (2014a, 2014b) reported that the incidence and severity of 'flesh browning' in 'Rojo Brillante' persimmon was greater the longer the CO_2 exposure time taken to remove astringency and suggested the implication of oxidative stress in this postharvest disorder. Gorny et al. (2002) also reported CO_2 injury in fresh-cut pears when similar controlled atmospheres were applied (air + 10% CO_2 and air + 20% CO_2). Later studies confirmed that although the combination antioxidants-active MAP in 5kPa O_2 was not necessary to accomplish a

9-day commercial shelf life, it helped improve the visual quality of fresh-cut 'Rojo Brillante' persimmons, showing a synergic effect of both technologies. In some fresh-cut fruits and vegetables, a rapid establishment of low O_2 and/or elevated CO_2 environment (active MAP) is considered critical for the preservation of cut surface browning, since the process accelerates attainment of the equilibrium concentrations and lessens the PPO activity at the initial stage (Toivonen et al., 2009). Wright and Kader (1997a; 1997b) studied the effect of processing and controlled atmosphere storage on the quality of fresh-cut 'Fuyu' persimmon. In this cultivar, the visual quality of persimmon was improved with the storage under 2kPa O_2 + 12 kPa of CO_2 atmosphere and persimmon slices reached 8 commercial days at 5 ºC.

In the process to develop optimum antioxidant edible coatings for fresh-cut 'Rojo Brillante' persimmons, the initial work consisted on the study of different biopolymer matrix (whey protein isolate (WPI), soy protein isolate (SPI), hydroxypropyl methylcellulose (HPMC) or pectin) amended with the antioxidant combination selected in previous works of this Thesis. These polymers were selected as they proved effective to control enzymatic browning in other fresh-cut commodities (Pérez-Gago et al., 2005a; Shon and Haque, 2007; Ghidelli et al., 2010). The coatings and the antioxidant solution reduced enzymatic browning of fresh-cut 'Rjo Brillante' persimmon; but there were not significant differences between them, which indicates that the effect to reduce enzymatic browning of persimmon slices was due to the presence of the antioxidants in the different edible coating formulations. Nevertheless, the application of the HPMC- and the pectin-based coatings helped extend the commercial shelf life of persimmon fruits to 7-9 days at 5 ºC, depending on the MS, by reducing the incidence and severity of 'flesh browning' in persimmon slices. Although many works report the beneficial effect of edible coatings to reduce enzymatic browning of fresh-cut commodities, very little is known about the contribution of coatings with no active ingredients to enzymatic browning. In this sense, our results provide an insight of the effect of different biopolymer matrixes (proteins and polysaccharides) in this fresh-cut commodity and open the need to study how to improve coating functionality, as well as to understand the effect of the coatings on other physiological changes in processed fruit.

On the other hand, whereas the incorporation of the antioxidants to the different edible coatings did not affect the colour parameters of fresh-cut persimmons compared to the antioxidant aqueous solution, the addition of the different antimicrobial agents (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}) to the antioxidant pectin-based coatings did affect them, which reflects the importance of minor ingredients for the final performance of the coatings. Thus, the coating with 2 g kg^{-1} PS was the most effective to maintain high hue angle values in persimmon tissue over 7 storage days at 5ºC and 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. On the other hand, NI did not affect the colour parameters in persimmon tissue compared to the antioxidant solution.

Finally, the combination of active MAP (5 kPa O_2) and the antimcrobial edible coating (pectin - 10 g L^{-1} CA - 10 g L^{-1} $CaCl_2$ - 500 UI/mL NI) significantly reduced initial enzymatic browning compared to those samples stored in air conditions, which helped improve the visual quality of coated persimmon slices. Overall, the judges scored the combination of coated and active MAP samples with a value of 7 (very good) during the 9 days of storage, whereas the application of each one 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. These results are also in agreement with the behaviour observed in our previous work with the combination antioxidants-active MAP and place the combination of both technologies as an effective way to reduce enzymatic browning, to improve the visual quality and to extend the shelf life of 'Rojo Brillante' persimmon. In other works, the combination of a soy protein-antioxidant coating with the active conventional MAP (5 kPa O_2 + 15 kPa CO_2) also showed a synergic effect in controlling tissue browning of fresh-cut 'Rojo Brillante' persimmon. However, the application of these technologies established the limit of marketability by day 8 of storage (Ghidelli et al., 2010).

The effect of the edible coatings to reduce enzymatic browning also correlated with a lower PPO activity. On the other hand, many works report a reduction in respiration rate of fresh-cut fruits by coating application (Rojas-Graü et al., 2007; Fontes et al., 2008; Raybaudi-Massilia et al., 2008). However, in our work the effect of the pectin-based edible coating to reduce respiration rate of persimmon slices was only observed in samples packed under active MAP. This effect could also explain the synergic effect of both technologies to reduce enzymatic browning.

3. Prevention of texture loss in fresh-cut 'Rojo Brillante' persimmon

Softening or loss of tissue firmness is a quality defect that compromises the shelf-life of many fresh-cut fruits and particularly of 'Rojo Brillante' persimmon. As discussed above, the selection of appropriate firmness, given by the MS at harvest and/or postharvest ripening, appears as a crucial factor to maintain good quality of minimally processed persimmons during processing and storage at 5 ºC.

In addition to MS, the antioxidant treatment was the factor that contributed the most to the loss of firmness in fresh-cut persimmons. Thus, the most effective antioxidants to reduce enzymatic browning (AA and CA) significantly reduced the firmness of persimmon slices compared to control samples. Furthermore, these differences were more important in fruits harvested in the first half of the season (MS1) than in late season persimmons (MS2), with the latest having an important firmness drop in all the treatments. Reduced fruit firmness by acid solutions has also been reported in some fruit tissues, which indicates some damage of the cell wall structure. Thus in fresh-cut pears, the application of AA reduced firmness by up to 20%, whereas a 5% reduction was reported in the control samples (Oms-Oliu et al., 2006).

The most common treatment used to reduce softening of minimally processed fruits is to dip fruit pieces in calcium dips (Garcia and Barrett, 2005). In our work, the application of 5 g L^{-1} $CaCl_2$ dips was not effective enough to maintain fresh-cut persimmon firmness, probably because calcium levels were too low. However, the combination of either CA (5 or 10 g L^{-1}) or AA (10 g L^{-1}) with 10 g L^{-1} $CaCl_2$ helped maintain

the firmness of the persimmon slices within the same range as the control samples during storage at 5 ºC in fruits harvested at the beginning and at the end season. The combination 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ also resulted effective to maintain fruit firmness in later works, where antioxidant treatments were combined with MAP and edible coatings to extend the shelf life of fresh-cut 'Rojo Brillante' persimmon. Therefore, the results confirm the beneficial effect of 10 g L^{-1} $CaCl_2$ to prevent the firmness loss of fresh-cut persimmons that occurs when fruits are treated with acidic antioxidants to control enzymatic browning.

Nowadays, the use of 1-Methylcyclopropene (1-MCP) has become a common practice in the packing houses to prolong the storage time and to extend the campaign of 'Rojo Brillante' persimmon. In this sense, the postharvest application of 1-MCP helped maintaining the firmness in whole fruit during 45 days of storage at 1 ºC. However, after 60 days of storage at 1 ºC no differences were observed between 1-MCP treated and untreated samples. This allowed to process fresh-cut persimmons after storage at 1 ºC with good firmness, as the samples that were initially treated with 1-MCP had higher firmness than those untreated. Several woks have also reported the beneficial effect of 1-MCP to retain firmness in fresh-cut fruits such as apple (Perera et al., 2003; Calderón-López et al., 2005), pineapple (Buda and Joyce, 2003), kiwifruit, mango and persimmon (Vilas-Boas and Kader, 2007), melon (Ergun et al., 2006), and pears (Lu et al., 2009).

It is well known the effect of controlled atmospheres and MAP with high CO_2 and low O_2 to reduce softening during postharvest storage of fruits and vegetables, which has been attributed to the reduction of cell-wall-degrading enzymes activity as well as of the metabolic activity of the product as respiration rate is reduced (Toivonen et al., 2009). However, they have also been described negative effects. Thus for example, the application of 4 kPa O_2 + 5 kPa CO_2 delayed firmness loss in fresh-cut pineapple (Pan et al., 2015) and similar atmospheric conditions positively influenced firmness in fresh-cut apples compared to air conditions (Cortellino et al., 2015). However, high CO_2 levels caused softening and electrolyte leakage in cut peppers (López-Gálvez et al., 1997) and low O_2 and high CO_2 (10-20 kPa) MAPs did not prevent softening of fresh-cut pears (Gorny et al., 2002), bananas (Vilas-Boas et

al., 2006) or apples (Rojas-Graü et al., 2007). In our work, the use of controlled atmosphere with high CO_2 concentrations induced tissue softening of persimmon slices if compared to the samples stored in the air conditions. When low O_2 controlled atmospheres were applied, fruit firmness decreased at a similar rate as the samples placed in atmospheric conditions, showing no effect of these atmosphere in maintaining firmness of persimmon slices. These behaviours were observed in both water and antioxidant-dipped samples. When persimmon slices were packed in active MAP (5 kPa O_2) and passive MAP, the effect of active MAP to reduce firmness loss of fresh-cut persimmons was observed only in the control samples; whereas those dipped in antioxidant solution (1% CA + 1% $CaCl_2$) were not affected by packaging conditions and showed a lower firmness.

As with other technologies, the effect of edible coatings to reduce firmness loss depends on many factors, such as coating composition, commodity, storage conditions, MS, etc, as reflected by many works in the bibliography. In this Thesis, the antioxidant pectin-based edible coating had little or no effect on maintaining the firmness of fresh-cut persimmons. Only in persimmons processed at the beginning of the season (MS1), the SPI- and HPMC-coated samples present significantly higher firmness values after 9 days of processing than the rest, although no differences were observed for the other storage days. When the antimicrobial agents were added to the pectin-based edible coatings, fruit firmness was not affected and 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. The pectin-coated samples amended with NI were not benefited by active MAP with 5 kPa O_2 and the application of active MAP only helped to retain firmness of uncoated samples, which confirmed previous work where active and passive MAP were studied.

Overall, the results confirm that 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 and the addition of $CaCl_2$ also seems necessary when acidic antioxidants are used to control browning in order to minimise the softening of persimmon slices.

4. Sensory quality

The most appealing attributes of fresh-cut products include their perception of freshness, taste and flavour, in addition to convenience. When assessing quality, consumers take product appearance into consideration as a primary criterion. Appearance can be characterized by size, shape, colour, gloss, and absence of defects (Garcia and Barrett, 2005). However, in fresh-cut commodities colour and absence of defects contribute more than any other factor and generally determine the commercial shelf life (i.e. limit of marketability). For this reason, visual appearance has been discussed together with browning control of fresh-cut 'Rojo Brillante' persimmon.

While appearance may have a major impact on consumers first buy, other sensory attributes as taste, aroma, and texture are what will ensure consumer's repeated purchases of the fresh-cut commodity (Garcia and Barrett, 2005). In this Thesis, the taste evaluation included the overall flavour or taste, presence of off-flavours, and firmness of the fresh-cut 'Rojo Brillante' persimmons. Overall flavour was rated on a 9-point scale, where 1-3 represented a poor quality range, 4-6 an acceptable quality range, and 7-9 an excellent quality range. 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, 3 = neither firm nor soft, and 5 = very firm.

In all the studies of this Thesis, persimmon fruits were evaluated as having an excellent overall flavour at harvest (evaluated in the range 7-8) independently of the MS or the storage time at 15 ºC before processing, which confirm that the fruits were harvested as having the characteristic flavour of a perfectly ripe fruit. Nevertheless, fruits harvested in the second half of the season were generally scored slightly better than fruits harvested in the first half.

After processing and storage at 5 ºC, the overall flavour of the persimmon slices decreased, indicating that some flavour components may disappear. Nevertheless, the scores remained within the range of acceptability (4-6) at the end of the different storage periods at 5 ºC and depended on the treatment. Thus, the application of 10 g L^{-1} AA or CA and 5 g L^{-1} $CaCl_2$ did not affect the overall flavour of the fresh-cut 'Rojo Brillante' persimmons, which were evaluated as the control samples, and

did not give rise to any off-flavour in the samples. However, the application of 5 g L^{-1} Cys induced a slight off-flavour in the samples, and samples were evaluated with the lowest flavour quality at the end of the 8 days storage. Richard-Forget et al. (1992) reported that the application of Cys is often incompatible with product taste due to the formation of sulphur compounds, as observed in the present work. When AA or CA were combined with 10 g L^{-1} $CaCl_2$, the overall flavour was not affected and remained within the range of acceptability at the end of the 9 storage days at 5 ºC and did not give rise to any off-flavour in the samples. However, when persimmons were stored in active MAP (5 kPa O_2), the judges reported an acidic taste of samples dipped in 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$, which was also reflected in a slight off-flavour or atypical flavour by storage day 2. Nevertheless, the differences among treatments disappeared after 4 storage days and did not affect the overall flavour, which was evaluated as 6 (within the range of acceptability) throughout the 9 days of storage at 5 ºC.

When the antioxidants were incorporated into the different edible coatings, the overall flavour of persimmon slices also remained within the range of acceptability for 7 storage days at 5 ºC, which indicates that the coatings did not negatively affect sensory quality. This correlated with the absence or very slight presence of off-flavours in the samples, with no significant differences among treatments. The combination of antioxidants and antimicrobials in the pectin-based coating conferred slight acidity to samples, as reported by the judges, which did not correspond to the typical persimmon flavour. 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 the 9 days of storage, only the persimmon slices treated with the pectin-4 g kg^{-1} SB coating obtained a higher score for the 'off-flavour' attribute than the remaining samples, while no differences were found among the other treatments and the controls. Despite these results, all the treatments were evaluated with an overall flavour within the limit of acceptability (5-6 range) during the whole storage period. Similarly, the combination of the NI-based antimicrobial edible coating with active MAP imparted a very slight off-flavour (scored as 2) to persimmon slices after 7 days of storage, but at the end of the 9 days of storage all the persimmon slices

were evaluated with an 'overall flavour' within the limit of acceptability (5.6-7.0 range). Therefore, these results indicate that, although some flavour components may disappear during processing or in other cases off-flavour may develop due to the different treatments applied, 'Rojo Brillante' persimmon might be processed during 9 days of storage at 5 ºC with an acceptable overall flavour. In any case, off-flavour values below 2 compromised the overall acceptability of the persimmon slices, given by flavour scores above 5.

As described above firmness is a crucial quality attribute in fresh-cut persimmon that, beyond the instrumental analysis, requires a sensory analysis to establish the effect of textural changes in the consumer's perception. In this Thesis, the factors that affected the most the persimmon firmness were MS at harvest, the treatment with AA or CA, and the time of storage at 5 ºC after processing. In general, sensory firmness confirmed the instrumental texture analysis results as it showed a similar trend. Overall, a high firmness at harvest helped maintaining a good sensory firmness during storage and only those samples that had an instrumental firmness close to 10-15 N were evaluated as soft at the end of storage at 5 ºC. This confirmed the work by Romaguera et al. (2009) that correlated sensory and instrumental firmness of different persimmon cultivars during storage. In their work, instrumental firmness above 30 N corresponded to sensory scores of 4-5 (firm-very firm), values that fell within the 20-30N range corresponded to sensory scores of 3 (neither firm nor soft), and values below 10 N indicated the limit at which persimmon fruits were scored by the sensory panel as being soft.

5. Nutritional aspects

Persimmon fruits are generally recognized as an outstanding source of biologically active compounds related to both nutritional and nutraceutical values (Giordani et al., 2011). The nutritional relevance of persimmon fruit is due to their high content in vitamin C and provitamin A carotenoids (β-carotene and β-cryptoxanthin), whereas the nutraceutical value is mainly related to dietary carotenoids (β-carotene, β-cryptoxanthin, zeaxanthin, lutein, and lycopene) and the high content in condensed tannis which have been implicated in the reduction of

degenerative human disease due to their antioxidant and free radical scavenging properties (de Ancos et al., 2000; Chen et al., 2008).

In general terms, processing, storage at 5 ºC after processing, application of antioxidant treatments or edible coatings, did not affect the total vitamin C of fresh-cut 'Rojo Brillante' persimmon, although some significant differences among treatments were observed. In the case of controlled atmospheres, the fruits processed in the second half of the season (MS2) obtained higher values for total vitamin C than those processed in the first half (MS1). In all cases, the concentrations obtained herein ranged from 150 mg/100 g fresh weight (FW) to 200 mg/100 g FW and few cases fell beyond this range. These values fell within the same range as those obtained in other studies for non astringent persimmon cultivars and astringent cultivar 'Rojo Brillante' at similar harvest periods (Wright and Kader, 1997a; Del Bubba et al., 2009; Giordani et al., 2011). The variability observed in vitamin C values during storage at 5 ºC of fresh-cut 'Rojo Brillante' persimmon was also reported in fresh-cut 'Fuyu' persimmon by Wright and Kader (1997a). These authors reported a loss in total vitamin C on the first day after cutting, but values then recovered to levels that were not significantly different from the first day. For other commodities, the effect of cutting and storage at 5 ºC on total vitamin C was variable. Gil et al. (2006) reported an increased total vitamin C during storage at 5 ºC for 9 days for pineapple pieces and strawberry slices, but it lowered in fresh-cut mangoes, cantaloupes, watermelons and kiwi fruits.

Several authors have reported variations in the antioxidant capacity in plants induced by abiotic stress conditions. Prior et al. (1998) found increased antioxidant capacity, total phenols and anthocyanins with more advanced maturity at harvest in a wide variety of berries. The same results have been observed in peppers, apricots and cucumbers (Navarro et al., 2006; Hegedüs et al., 2011; Sudha et al., 2011). In the first and third chapters of this work, the antiradical capacity of 'Rojo Brillante' persimmons increased as the MS or the storage time of whole fruit at 15 ºC increased (given by lower EC_{50} values). However, in the second chapter, the fruits harvested at the beginning of October (MS1) presented greater radical scavenging activity than those harvested in mid-November (MS2). Del Bubba et al. (2009) investigated changes in

radical scavenging activity during the growth and maturation of 'Kaki Tipo' and 'Rojo Brillante' persimmon. In the final maturation stage, between mid-October and mid-November, free radical scavenging activity decreased in 'Kaki Tipo', whereas 'Rojo Brillante' persimmon showed erratic behaviour with a saw tooth pattern. In the present Thesis, a wide variability in the EC_{50} values was also recorded at harvest and during storage at 5 ºC and values ranged in general between 100 and 450 g sample per Kg of $DPPH^{\bullet}$. Therefore, some of the differences observed might be due to the natural heterogeneity of fruit. Furthermore, this variability also leads to think that the differences observed in the antiradical capacity of fresh-cut 'Rojo Brillante' persimmon in the different studies might not be due to the treatments applied (i.e. antioxidant dips, edible coatings, or controlled atmosphere storage), but to other factors.

A wide range of variability has been described in the bibliography for total phenolic content of persimmon fruits on the basis of cultivar, environment effects, and even the different applied extraction methods (Giordani et al., 2011). In this Thesis, total phenolic content of 'Rojo Brillante' persimmon at harvest ranged between 5 and 10 mg gallic acid per 100 g FW. These values are similar as those reported for other non-astringent cultivars such as Hiratanenashi and Tonewase. In astringent cultivars, however, total phenolic content is generally five to forty times higher than in the non-astringent group. However, after astringency removal values significantly drop as reported in 'Rojo Brillante' and 'Kaki Tipo' due to the insolublization of soluble tannins (Del Bubba et al., 2009; Giordani et., 2011). According to Del Bubba et al. (2009) the total soluble phenolic concentration of fresh 'Rojo Brillante' dropped from 220 mg gallic acid equivalents per 100 g FW in astringent fruit to 31 mg gallic acid per 100 g FW after removal of astringency with CO_2. In general, the total phenolic content displayed the same trend as radical scavenging activity, as phenolic compounds contributed in a large extend to the antioxidant activity of fruits and vegetables (Kaur and Kapoor, 2002). In this sense, the total phenolic content in chapter 1 was significantly greater in the samples processed in the second half of the season (MS2) than those processed in the first half (MS1); whereas the opposite was observed in the rest of the studies. Similarly, chapter 1 showed an increase in total phenolic content after processing and during

storage at 5 ºC that was not observed in later works. In other fresh-cut fruits, phenolic content varied depending on the fruit and processing conditions. Thus, Gil et al. (2006) reported a moderately reduced phenolic content during storage at 5 ºC in mango and cantaloupe cubes, whereas phenolic content was maintained in fresh-cut kiwi fruit, strawberry or pineapple over 9 storage says. Reyes and Cisneros-Zevallos (2003), on the contrary, reported that potato wounding induced the synthesis of phenolic compounds, which translated in higher total phenolic content. On the other hand, it could be concluded that the total phenolic content of fresh-cut 'Rojo Brillante' persimmon was not affected by the antioxidant treatments, the controlled atmospheres or the edible coatings studied in this Thesis and the differences observed among total phenolic values could be attributed more to biological variation than to an effect of the treatments.

Eight carotenoids were identified in 'Rojo Brillante' persimmon: α-carotene, β-carotene and six xantophylles (trans-viloaxanthin, cis-violaxanthin, lutein, zeaxanthin, α-criptoxanthin and β-criptoxanthin). Of these, the major carotenoids were β-cryptoxanthin and β-carotene, followed by α-cryptoxanthin and α- carotene. Other works have indicated that β-cryptoxanthin and β-carotene, together with lycopene, are the main carotenoids in 'Rojo Brillante' persimmons (de Ancos et al., 2000; Plaza et al., 2012). The high lycopene content reported in 'Rojo Brillante' persimmon by other authors can be attributed to a more advanced MS of the fruit. The relative importance of β-cryptoxanthin and β-carotene differed in the different studies of the Thesis. Some works have described β-cryptoxanthin as the major carotenoid in 'Rojo Brillante' persimmon (De Ancos et al., 2000; Plaza et al., 2012). This was confirmed in Chapter 1, but not in the later works. This variability in the contribution of the different carotenoids could be attributed to the maturity stage or to the different growing conditions of the fruits. Thus, the effect of MS at harvest (i.e. the more advanced the MS stage, the higher the total carotenoid concentration) and storage time at 15 ºC before processing (i.e. carotenoid content increased with storage time at 15 ºC) on carotenoid content of 'Rojo Brillante' persimmon was observed in the fruits harvested with the more advanced MS in this Thesis (i.e. CI of 17.6 ± 1.8 and firmness of 20.9 ± 5.4 N).

In general, carotenoids of fresh-cut persimmons were not affected by antioxidant treatment, controlled atmosphere storage, edible coating application, or by storage at 5 ºC. Similarly, no effect of processing on carotenoid content has been reported in Fuyu persimmon slices, fresh-cut papayas (Wright and Kader 1997a; Rivera-López et al., 2005), kiwifruits and strawberries (Gil et al., 2006).

By considering the importance of β-cryptoxanthin and of β carotene as provitamin A, the retinol equivalent (RE) was also calculated. Overall, the RE of 'Rojo Brillante' persimmon ranged between 20 and 27 μg/100 g FW and only in the more advanced MS, given by high colour and low firmness, the RE reached values as high as 65 μg/100 g FW. These values are of the same order of magnitude as 15 other persimmon cultivars reviewed by Giordani et al. (2011). In 'Rojo Brillante', Plaza et al. (2012) also reported RE values of around 22 μg/100 g FW in fruits with a similar maturity stage as those used in our studies and de Ancos et al. (2000) obtained values of around 77 μg/100 g FW for fruits harvested in a more advanced ripening stage.

6. Microbial spoilage

Although it is considered that whole fresh produce, for the most part, are among one of the safest foods, the safety of fresh-cut products requires a great deal of attention. The processing that fruit undergo turns them more vulnerable to microbiological risks, and the sensory quality is meaningless if the product is unsafe. Therefore, special care should be taken from farm to retail storage to minimize microbial spoilage and growth (Garcia and Barrett, 2005).

'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). Under the studied conditions, growth of moulds, 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. However, the counts of total aerobic mesophilic bacteria significantly increased in control samples during storage. Immersion in the antioxidant solution (10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$) or the pectin-based coatings amended with the different

antimicrobial agents (PS, SB or NI) effectively maintained or reduced the growth of mesophilic bacteria. The application of the antioxidant aqueous solution was as effective as the pectin-based edible coatings amended with 2 or 4 g kg^{-1} PS, which can be attributed to a reduction in the pH. Thus, 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 (Aguayo et al., 2008; Freitas et al., 2013). 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. 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). In organic acid salts, optimal antimicrobial activity has occurred at low pH values, when the undissociated form is present. Therefore, the addition of CA might have contributed to enhance the antimicrobial activity of these organic acid salts by lowering the pH of the formulations to pH values of 2.6, which fall within the optimum range for these salts (Valencia-Chamorro et al., 2011a). Similarly, the 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 (Ross et al., 2003). These results were confirmed in the second work, where the pectin-NI based edible coating effectively controlled the growth of mesophilic bacteria of fresh-cut persimmon after 8 storage days, independently of the packaging conditions (active MAP or air conditions). However, at the end of storage uncoated samples packed in the active MAP (5 kPa O_2) had 1 log reduction of mesophilic bacteria compared to those packed in air conditions. 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 microorganism in fresh-cut

pears, melon, honey pomelo, and mushroom slices, among others (Simón et al., 2005; Oms-Oliu et al., 2008; Li et al., 2012) and reduced the development of aerobic psychrophilic bacteria and *Pseudomonas* in leaf spinaches (Tudela et al., 2013).

Post-processing contamination or recontamination of the surface of food products by pathogenic microorganisms has led to recalls and outbreaks of food-borne illness. 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). Considering that 'Rojo Brillante' persimmon is described as a low acidity fruit with a pH around 6, the effect of antimicrobial pectin-based coatings on the growth of *E. coli*, *S. enteritidis* and *L. monocytogenes* in artificially inoculated fresh-cut 'Rojo Brillante' persimmons was also assessed. The application of coatings amended with PS, SB or NI lowered the initial populations (just after coating, AT) of *E. coli* and *S. enteritidis*; whereas the population of *L. monocytogenes* was reduced only by the pectin-NI edible coating. On storage day 8, only the antimicrobial coatings 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 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 and the population of *L. monocytogenes* maintained the 4.0 $\log_{10}$ units reduction observed after coating application.

These results revealed the potential growth-inhibition effect of NI added to the pectin-based edible coating in order to reduce populations of *E. coli*, *S. enteritidis* and *L. monocytogenes* in fresh-cut 'Rojo Brillante' persimmon. Therefore, this coating was tested in combination of active MAP storage (5 kPa O_2) in a later work to extend the shelf life of fresh-cut persimmon. In this case, the coating was more effective on reducing the *E. coli* and *S. enteritidis* populations for both packaging conditions (active MAP and air) than in the previous work and achieved a complete inhibition of *E.coli* and *S. enteritidis* in the samples under MAP

(Coating-MAP) or air conditions (Coating), respectively, after 8 storage days. Furthermore, the impact of the coating upon the population of *L. monocytogenes*, although significant compared to uncoated samples, was lower than in the previous work, with only 2.0 $\log_{10}$ units reduction in the samples packed in air; whereas the combination coating-MAP reached more than 5.0 $\log_{10}$ units reduction by the end of the storage period.

The effectiveness of NI to inhibit 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). For example, NI inhibited the growth of *L. monocytogenes* in processed mangoes or melon (Teixera-Barbosa et al. 2013; Ukuku and Fett, 2004). However, some works have described a resistance of NI to *L. monocytogenes*, 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 (Davies and Adams, 1994; Mazzotta and Montville, 1997; Nawrocki et al., 2014). Nevertheless, the differences observed in both works could be due to other factors, since the population of *L. monocytogenes* of 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 such as *E. coli* and *S. enteritidis*. 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 as 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 in *in vitro* studies (Stevens et al., 1992; Cutter and Siragusa, 1995). Therefore, the low pH of the pectin-based edible coating (pH 2.30) might have enhanced the effect of NI against the different food-borne pathogens tested.

Overall, the results of this Thesis indicate that 'Rojo Brillante' persimmon can be processed as a fresh-cut commodity by using an integrated approach that would consider the quality of the raw product

and an appropriated combination of technologies. Thus, the combination of low temperature storage, active MAP (5 kPa O_2) and the antimicrobial/antioxidant edible coating (pectin - 10 g L^{-1} CA - 10 g L^{-1} $CaCl_2$ - 500 UI/mL NI) provides to producers and consumers a product with sufficient shelf life and the maxima safety and quality. This combination maintains a very goof visual quality of fresh-cut 'Rojo Brillante' persimmon over 9 storage days, without affecting negatively other qualities attributes.

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CONCLUSIONS

GENERAL CONCLUSIONS

1. The selection of an adequate maturity stage (MS) at harvest before processing is a determinant for the commercial shelf life of fresh-cut 'Rojo Brillante' persimmons as it affects firmness and enzymatic browning.

2. The use of 10 g L^{-1} ascorbic acid (AA) or 10 g L^{-1} citric acid (CA) controlled tissue browning and maintained the general visual quality of fresh-cut persimmons above the limit of marketability by up to 6-8 days of storage at 5 ºC, depending on the MS. However, these antioxidants reduced fruit firmness as compared to control samples

3. 'Rojo Brillante' persimmons harvested at the beginning of the season can be processed as a fresh-cut commodity, even after 3 days of storage at 15 ºC if treated with AA or CA. Whereas in late season fruit, processing the fruits after harvest and being treated with AA are recommended.

4. Selecting fruits with good firmness and the addition of 10 g L^{-1} $CaCl_2$ helped prevent loss of the firmness of fresh-cut 'Rojo Brillante' persimmons treated with acidic antibrowning agents. Overall, the combination of 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ can be used as antibrowning treatment to commercialize persimmon 'Rojo Brillante' as a fresh-cut commodity for up to 7-8 days at 5 ºC.

5. The application of 1-MCP at harvest reduced softening of whole 'Rojo Brillante' persimmon during storage at 1 ºC and allowed to process fruit up to 45 days under commercial packaginghouse conditions.

6. Controlled atmospheres with high O_2 (21 kPa) and elevated CO_2 (10 or 20 kPa) did not prevent enzymatic browning and softening of fresh-cut 'Rojo Brillante' persimmons. Furthermore, high CO_2 concentrations induced 'flesh browning' on the tissue.

7. Application of antioxidants (CA-$CaCl_2$) in combination with active modified atmosphere packaging (MAP; 5 kPa O_2) controled tissue browning and maintained the general visual quality of fresh-cut persimmons within the limit of marketability for 9 storage days at 5 ºC.

8. Antioxidant edible coatings based on whey protein isolate (WPI), soy protein isolate (SPI), hydroxylpropyl methylcellulose (HPMC) and pectin proved effective to control enzymatic browning of fresh-cut persimmon, being the HPMC- or pectin-based coatings the most effective to extend the commercial shelf life.

9. Antimicrobial pectin coatings containing 2 g kg^{-1} potasium sorbate, 4 g kg^{-1} sodium benzoate or 500 IU mL^{-1} nisin (NI) controlled enzymatic browning and reduced the total aerobic mesophilic bacteria of fresh-cut 'Rojo Brillante' persimmon, which accomplished a commercial shelf life of 7 days at 5 ºC.

10. The combination of active MAP (5 kPa O_2) and the antimcrobial pectin coating, formulated with the 10 g L^{-1} CA + 10 g L^{-1} $CaCl_2$ and 500 IU mL^{-1} NI, proved to be the most effective technology to maintain the visual quality of persimmon slices during storage at 5 ºC, being evaluated as 'very good' at the end of the 9 storage days, without affecting negatively the overall flavour.

11. The coating formulated with the combination of the antibrowning agents (CA-$CaCl_2$) and 500 IU mL^{-1} NI effectively stunted the growth of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* in artificially inoculated fresh-cut persimmons.

12. Nutritional quality (vitamin C, free radical scavenging activity, total phenolic content, and carotenoids) of fresh-cut 'Rojo Brillante' persimmon was not negatively affected by processing (cutting and storage at 5 ºC), antioxidant dips, controlled atmosphere storage or edible coatings. Free radical scavenging activity and total carotenoid content increased in late-season persimmons.