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Effects of combinational use of controlled atmosphere, cold storage and edible coating applications on shelf life and quality attributes of fresh-cut persimmon fruit

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Abstract

Background: Persimmon fruits are cherished for their unique flavor and high nutrient content. In the present study, the effects of *Aloe vera*-based edible coatings (EC) added with 1% ascorbic acid, 1% citric acid and 5% calcium chloride in modified atmosphere packaging (MAP) and their impact on shelf-life quality of fresh-cut persimmon fruit, were investigated. The experimental design consisted of four treatments 1) *Aloe vera* edible coating + modified atmosphere packaging (EC + MAP), 2) untreated fruit packaged with MAP (CTR + MAP); 3) *Aloe vera*-based edible coating in passive atmosphere (EC PASSIVE); 4) untreated fruit in passive atmosphere (CTR PASSIVE). Persimmon fruit were stored at 5 °C for 3, 6 and 9 days. At each storage time, firmness, weight loss, sugar content, organic acids, polyphenol oxidase (PPO), browning index respiration rate, sensory and microbiological analysis, were investigated.

Results: Our results were confirmed also by the sensory analysis in which both EC-treated fruit scored the highest values for positive descriptors. EC + MAP treatment showed the most effective result in maintaining total carotenoids, ascorbic acid, glucose and $\rm CO_2$ inside packaging reducing the PPO activity and the flesh browning of persimmon slices. EC + MAP treatment controlled the growth of total mesophilic microorganisms, pseudomonads, *Enterobacteriaceae*, yeasts and molds.

Conclusions: The obtained results confirmed the importance of coating composition in controlling post-harvest decay and maintaining fruit quality.

Keywords: Diospyros kaki, Glucose, Succinic acid, Carotenoids, PPO, Browning

Introduction

Persimmon (*Diospyros kaki* L.) is cultivated in China, Spain and Italy, its fruits are cherished for their unique flavor and high nutrient content. Since persimmons are climacteric fruits, ethylene plays a major role in the regulation of ripening [1]. In Europe, cv 'Rojo Brillante'

is an astringent variety characterized by good growing conditions, excellent color, caliber, sensory characteristics, and good nutritional properties. Persimmon ripens between late October and early November. Flesh softening reduces the harvest window and causes excessive persimmon fruit drop. After harvest, high levels of CO_2 are applied to remove astringency while firmness is preserved [2]. Harvest time is essential for the quality of minimally processed persimmon fruit [3]. Indeed, bioactive compounds content in fruit (citric, ascorbic acid, phenols and sugar content) could

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change after cut due to exposition at O_2 , or increase of respiration rate and microbial groups [4, 5].

Processed fresh-cut fruits are characterized by a high perishability caused by cut. The high polyphenol oxidase (PPO) activity, and therefore the browning of tissues followed by an organoleptic and nutritional loss, could be issues that lead to the non-marketability of the product and compromise the hygienic quality health. PPO is a well-known enzyme responsible for tissue browning in mechanically injured fruits [6]. PPO catalyzes the oxidation of phenolic compounds to *o*-quinones which subsequently polymerize to form dark-colored pigments [7].

Chemical antibrowning included into active coatings can both inhibit PPO activity and offer a strong barrier for oxygen passage, resulting in enzymatic browning in coated items being delayed. Ascorbic acid, citric acids, phenolic acids, and resorcinols are chemical antibrowning commonly employed in coating formulations [8]. O-Quinones produced by PPO enzymes are reduced back to their phenolic substrates by ascorbic acid, which has antioxidant and antibrowning characteristics [9].

For this reason, many technological innovations have been implemented to preserve organoleptic and nutritional losses and extend shelf life such as the combination of edible coating, browning agents, and MAP (modified atmosphere packaging) to maintain the quality characteristics of minimally processed fruits. Indeed, several studies confirm that by reducing the concentration of oxygen and increasing the concentration of carbon dioxide, respiratory activity and ethylene production could be reduced slowing down the enzymatic reactions and some physiological alterations, and thus maintaining the quality of the product longer [9, 10]. Interesting results were obtained with the adoption of edible coatings (EC) which are innovative storage systems that exploit the solubility principle of some functional substances such as antioxidants or antimicrobials in a solvent, usually represented by water. These properties can be also improved by adding further substances, such as glycerol and CaCl₂, which allow the film to have better adhesion and structure [11] and ensure an efficient application on fruits. Recent studies carried out with Aloe gel have shown how its application during post-harvest is useful to preserve the qualitative characteristics [12]. The aim of this work was to evaluate the effects of the aloe edible composite coating containing natural antioxidant additives on physico-chemical, sensorial and nutritional quality of fresh-cut 'Rojo Brillante' persimmon fruit during 12 days of cold storage.

Materials and methods

Plant material

Persimmons (Diospyros kaki Thunb. cv Rojo Brillante) were introduced from Spain and grown in Italy. The orchard was planted in 2014 with a density of 556 trees per hectare at a spacing of 4.5 m between rows and 4.0 m on North-South rows. The trees were trained to free palmette and grafted on D. lotus rootstock. Fruits were harvested from a commercial orchard in Riesi (Sicily, Southern Italy, 350 m above sea level) at commercial maturity stage in November 2020, when fruits presented a firmness value of 58 ± 0.1 N. Three hundred fruits were formed to be exposed to CO2 treatment in closed containers (95% CO2 at 20 °C and 90% RH) for 24 h. After harvest, fruits were taken to the post-harvest laboratory of University of Palermo, where they were carefully selected basing on uniformity of size and color, and on lack of defects. Color, acidity and total soluble solid values were, respectively, L* 48.32 ± 6.82 ; a* -2.56 ± 1.20 ; b* following the CIELab, 0.1% of titratable acidity (expressed as percentage of malic acid) and 17.43 ± 0.05°Brix (total solid soluble content).

Experimental design

All fruits were brought to the laboratory and dipped in chlorinated water (100 ppm of free chlorine) for 360 s. 75–80 defective fruits (bruised, other physical damage, incorrect maturity, and anomalous color) were eliminated, and the remaining persimmon fruits were then selected by firmness ($5.3\pm0.3~{\rm kg~cm^{-2}}$) and average fruit weight ($250\pm1.5~{\rm g}$).

All utensils and surfaces were previously washed and sterilized. Temperature inside the laboratory was set at 4 °C to reduce bacterial proliferation and fruits were first washed with tap water and then immersed in chlorinated water (100 μ L L⁻¹) for 5 min, according to methodology reported by Allegra et al. [13]. Fruits were then air-dried for 2 min. Fruits were peeled and cut into 8 slices with a medium length of 4 cm with a sterilized stainless-steel knife.

Slices were placed in bi-oriented polystyrene (PS) bags (Carton Pack s.r.l., Rutigliano, Italy) before receiving any treatment. Fruit slices were dipped in different coating solution, for 60 s; the excess coating was drained, and the coated slices were dried in a forced-air dryer (20 °C) for 30 s.

Edible coating composition

Aloe vera-based gel preparation was carried out according to Zapata et al., (2013) [12]. One kg of leaves from Aloe plants were externally cleaned with a knife, deprived of the margin, and subsequently cut lengthwise; the

parenchyma was then separated from the epidermis. The gelatinous parenchyma obtained was blended with Ultra-turrax (Ultra-Turrax T25, Janke and Kunkle, IKa Labortechnik, Breisgau, Germany) for 5 min at 24.500 rpm, obtaining a gelatinous compound which was subsequently sieved to eliminate the fibrous portion. Then, 40% of the gel was removed and dissolved in 600 mL of distilled water and added with sodium alginate 0.5% w/v as gelling agent and glycerol 3% w/v to improve the viscosity and plasticity of the film. A further homogenization and a heat treatment at 90 °C for 40 min were applied to microbiologically stabilize the solution. A solution containing 1% w/v ascorbic acid to prevent further browning [14] and 1% w/v citric acid to maintain the pH value lower than 3, were added. After coatings application, fruits were treated with a 5% w/v CaCl2 solution in order to promote the formation of calcium pectates to reduce respiration rate and metabolic disorders which are responsible for qualitative decay [15].

The treatments consisted of:

- 1) CTR passive (untreated fruit packaged in passive atmosphere).
- 2) CTR+MAP (untreated slices+active atmosphere: 60 Kpa N_2 , 30 Kpa CO_2 and 10 Kpa O_2).
- 3) EC PASSIVE (edible coating slices packaged in passive atmosphere).
- 4) EC+MAP (edible coating+active atmosphere: 60 Kpa N_2 , 30 Kpa CO_2 and 10 Kpa O_2).

After being coated, *Rojo Brillante* persimmon fruits were stored in high-density polyethylene (HDPE) boxes in active and passive atmosphere at 5 °C and 95% relativity humidity (RH) for 9 days. Physicochemical properties, antioxidant activity, sensorial analysis and quality parameters were analyzed on 8 slices used for single replicates for each treatment at the beginning of the experiment immediately after coating (day 0), at 3, 6, and 9 day of storage (8 slices x 3bags \times 4 stages).

Respiration rate and ethylene production

Ethylene production and fruit respiration rate were measured immediately after harvest at 20 °C and moreover after storage at 5 °C for 72 h. Ten fruits were weighed with a digital scale and individually placed in 705-mL sealed glass containers, according to Crisosto et al. [16]. After harvest, ethylene production (μ L kg $^{-1}$ h $^{-1}$) was measured on integer fruits in a climatized chamber at 20 °C. According to previous literature, 1 mL of effluent air was taken with a syringe, as gas sample, from each respiration jar, and injected into a gas chromatograph (GC, Agilent Technologies 6890, Wilmington, Germany)

fitted with a FID detector and an alumina column F1 80/100 (2 m × 1/8 × 2.1, Teknokroma, Barcelona, Spain). The oven temperature was set at 140 °C while injector and detector were kept at 180 and 280 °C, respectively. The respiration rate was determined, with a portable gas analyzer (PBI Dansensor Checkpoint O_2 and CO_2) and expressed in mL CO_2 kg $^{-1}$ h $^{-1}$.

Sugar and organic acids determination

Soluble sugar and organic acids were extracted from fruits and analyzed by HPLC, according to the method described by Amorós et al. (2003) [17]. Five grams of persimmon mesocarp from each sample was homogenized with 10 mL of deionized water using a polytron homogenizer (IKA Labotechnik) and centrifuged at 10,000 RPM for 10 min. 10 mL of the supernatant was used to quantify sugars and organic acids by HPLC (Hewlett-Packard, series 1100, Waldbronn, Germany) chromatograph equipped with a SUPELCOGEL C-610H (30 cm 7.8 mm) column (at 30 C), a refractive index detector (for sugar analysis) and an UV-V detector (210 nm, for acid analysis). The elution system consisted of 0.1% H₂PO₄, running isocratically at a flow rate of 0.5mLmin⁻¹. Two separate extractions were made from each sample of 5 fruits and for each extraction sugars and organic acids were determined in triplicate.

The total soluble solids content (Brix) was detected by an ATAGO digital optical refractometer (Atago Co., Ltd, Tokyo, Japan). Titratable acidity (expressed as % malic acid) was determined by juice with 0.1 M NaOH to an endpoint of pH 8.10.

Color

The evolution of color change on the processed fruit was determined using the CIE L*a*b* system using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Japan). The relief was made at random on 4 slices of fruit in opposite parts, and the instrument has been calibrated using a standard white plate. L* indicates the brightness, a* indicates the chromaticity on an axis from green to red and b * the chromaticity on an axis from blue to yellow. To better evaluate the overall color variation, we proceeded by calculating the total color difference ΔE with the following formula: $\Delta E^* = (\Delta L^*2 + \Delta a^*2 + \Delta b^*2)^{\frac{1}{2}}$.

Firmness and weight loss

The difference in weight of each box was measured throughout the storage period using a digital scale of decimal precision (Gibertini, Novate Milanese, Italy) and the values were expressed as a percentage of weight loss:

Weight loss (%) = $[(W_i - W_d)/W_i] \times 100$,

where W_i is the initial weight and W_d is the weight recorded at each sampling date.

Weight of individual fruit was recorded immediately after treatment (day 0) and at each sampling date (3, 6 and 9 days of storage).

Browning index

The browning index was calculated according to Ruangchakpet and Sajjaanantakul [18] referring to the following formula:

Browning index (BI) =
$$[100 (x - 0.31)]/0.17$$
,

where
$$x = (a * + 1.75L *) / (5.645L * + a * -0.3012b *)$$
.

Sensory analysis

The sensory profile of fruit slices was evaluated by 11 semi-trained judges (6 females and 5 males, 22–45 years). Within the framework of preliminary meetings, 14 descriptors were selected according to the frequency of quotation (>60%) and used to define the sensory profile,: visual appearance (VA); compactness (C); sweetness (S); acidity (A); juiciness (J); astringent (AS); pungent (PU); fruit odor (FO); floury (FAR); off-odor (OFO); fruit flavor (FRF), alcoholic flavor (ALF), off-flavor (OFF) and overall evaluation (OVE).

Panel participants evaluated the intensity of each attribute on a discontinuous scale from 1 (absence of descriptor) to 9 (maximum descriptor intensity). The order of presentation of samples was randomized for each participant and water was provided for rinsing the mouth after every sample analysis.

Package O2 and CO2 analysis

 CO_2 and O_2 levels (kPa) were measured on each package at the beginning of each experiment and after 3, 6 and 9 days of storage, using a PBI Dansensor Checkpoint O_2 and CO_2 analyzer (Topac, Hingham, MS, USA) equipped with zirconium and infrared detectors, respectively.

Carotenoids content

Carotenoids were extracted from fruits and analyzed by HPLC, according to a method previously described by Xiong [19]. Components identification and quantitative analysis were carried out by an Agilent 1200 HPLC–DAD, equipped with a 5 μm C18m reverse phase column (250 mm \times 4.6 mm, Japan) and a 20 mm \times 4.6 mm C18 pre-column maintained at 35 °C, using external standard method. $\beta\text{-Carotenoid}$ and lutein compounds were detected at different stages. The standard samples of $\beta\text{-carotenoid}$ and lutein were purchased from Sigma-Aldrich Company (Ge). The concentrations of $\beta\text{-carotenoid}$ and lutein were calculated from the experimental peak area by analytical interpolation in a standard

calibration curve and expressed as mg 100 g⁻¹ of fresh weight.

Polyphenol oxidase (PPO)

Six fruit were peeled, cut in pieces of 2 cm², quick-frozen and stored at - 80 °C. Frozen samples (10 g) were homogenized in a blender for 30 s at maximum speed in 30 mL of ice-cold sodium phosphate buffer (100 mM, pH 6.4) with 0.5 g of polyvinylpyrrolidone (PVPP) to measure polyphenol oxidase (PPO, EC 1.10.3.2) [20]. PPO was assayed by incubating 0.5 mL of enzyme extract with 3 mL of catechol substrate (100 mM sodium phosphate, pH 6.4 and 50 mM catechol) and then it was monitored the change of absorbance at 398 nm for 10 s. The specific activity was expressed as Δ A398min⁻¹ mg⁻¹ protein. Protein content was assayed by the dye-binding method of Bradford [21] with bovine serum albumin as standard. Average values were calculated from the results of 6 measurements of different fruits.

Microbiological analysis

Microbiological analyses were carried out to evaluate the main microbial groups associated with A. vera coating, CTR and EC productions, and investigated for quality, hygiene, and safety aspects. Cell suspensions of A. vera coating samples were subjected to decimal serial dilutions in Ringer's solution, while solid (CTR and EC) samples (25 g) were first homogenized in 225 mL of Ringer's solution in a stomacher (Bag-Mixer 400; Interscience, Saint Nom, France) for 2 min at the highest speed and then serially diluted. The inoculation, cultivation, and incubation of the different microbial groups were as follows. Total mesophilic microorganisms (TMM) were spread plated on plate count agar (PCA) and incubated aerobically at 30 °C for 72 h. Members of the Enterobacteriaceae family were pour plated on double-layered violet red bile glucose agar (VRBGA) and incubated aerobically at 37 °C for 24 h. Pseudomonads were inoculated on Pseudomonas agar base (PAB) supplemented with 10 mg/ mL cetrimide fucidin cephaloridine, and incubated aerobically at 25 °C for 48 h. Eukaryotic microbial groups (yeasts and molds) were inoculated on yeast extract peptone dextrose (YPD) supplemented with 0.1 g/L chloramphenicol to avoid bacterial growth. For the determination of yeasts plates were incubated at 28 °C for 48 h, while for molds at 25 °C for 7 d. After incubation, colonies were enumerated, and the results were expressed as \log_{10} cfu/g of persimmon. Microbiological counts were carried out in duplicate. All media and supplements were purchased from Oxoid (Milan, Italy).

Statistical analysis

The experimental design on fresh-cut persimmon fruit consisted of three treatments and one untreated control, observed at 0, 3, 6 and 9 days after treatments. Eight slices were used as single replicates and analyzed at each sampling date for treatments and control. Analysis of variance was applied to collected data (Systat 13.0 for Windows was used as statistical software). Significant differences ($P \le 0.05$) were evaluated with Tukey's test.

Results and discussion

Ethylene and CO₂ production before treatments

Ethylene production of persimmon fruit is usually affected by maturity stage [22]. Ethylene production measured on *Rojo Brillante* persimmon fruit immediately after harvest time at 20 °C was 0.2 ± 0.1 mL kg⁻¹ h⁻¹ while a value of 0.09 ± 0.3 mL kg h⁻¹ was registered after storage for 72 h at 5 °C. At those stages, fruit respiration rate was 20.0 ± 1.5 and 5.3 ± 1.8 kg h⁻¹ CO₂, respectively.

Sugar concentration and total solid soluble (TSS)

The sugar concentration is very important to determine sensory quality and taste of fresh-cut persimmon fruit during storage time [23] (Table 1).

CTR Passive and CTR+MAP sucrose content showed a low decrease during the 9 days of storage, while in the other treatments, the mean values increase after 6 days of storage. After 9 days of storage EC + MAP reported the highest mean values. Glucose content increased in CTR PASSIVE and EC+MAP samples of 10.1% and 20.4%, respectively, while in CTR + MAP and EC PASSIVE treatments it decreased of 22.6% and 19.3% during storage. Furthermore, our results showed that EC+MAP samples slices had reached the highest mean values immediately after 3 days of storage. Glew et al. [24] showed that sucrose content remained relatively low and decreased during ripening while the glucose content reported a mean value of about 35.4 mg.100 g⁻¹ fw. The high value of glucose in EC+MAP could be due to a lower respiration rate of persimmon slices if compared to other treatments were an higher respiration rate could cause an higher consumption of glucose as substrate [4] (Figs. 8, 9). After cutting, fructose content remained stable in CTR PASSIVE treatment while the mean values of EC PASSIVE and CTR+MAP decreased until the 9th day of storage.

A different trend occurred in fructose content of EC+MAP treatment from 6 to 9 days of storage reporting significant differences unlike other treatments. Different results were reported by Zhang et al. [25] on persimmon fruit registering an increase of fructose during 30 days while on fruit treated with 1-MCP the fructose remained stable during storage. The TSS

Table 1 Sugar compositions (glucose, fructose and sucrose), in minimally processed persimmon fruit (*Diospyros kaki* L.), cv *Rojo Brillante* immediately after harvest (T0) and after 3, 6 and 9 days of storage

Time of storage	Sugar concentrations (µg/ mg)	CTR PASSIVE	EC + MAP	CTR + MAP	EC PASSIVE
TO	Sucrose	40.4 ns	40.4	40.4	40.4
	Glucose	271.5 ns	271.5	271.5	271.5
	Fructose	287.9 ns	287.9	287.9	287.9
	TSS (Brix)	17.1 ns	17.1	17.1	17.1
	Sucrose	38.9 ns	40.4	39.2	40.2
Т3	Glucose	289.0a	289.4a	269.6c	279.4b
	Fructose	210.3d	235.2c	266.3b	283.3a
	TSS (Brix)	17.1 ns	17.2	17.1	17.2
	Sucrose	38.6b	41.2a	38.9b	41.5a
Т6	Glucose	288.8b	300.2a	253.1c	266.4c
	Fructose	210.3c	221.2b	223.7b	271.2a
	TSS (Brix)	17.1 ns	17.2	17.2	17.3
	Sucrose	37.9c	48.6a	36.5d	41.2b
T9	Glucose	299.0b	327.4a	213.4c	214.6c
	Fructose	210.3b	247.5a	210.1b	219.1b
	TSS (Brix)	17.4a	17.5a	17.1ab	16.9b

At each sampling date, different letters indicate substantial changes between treatments *ns* non-significant

 $P \le 0.05$ was used in the Tukey's significant test

content did not show significant differences in all treatments but at 9th day EC PASSIVE reported the lowest mean values than other treatments.

Organic acids and titratable acidity content

Ascorbic, citric, malic and succinic acid were detected in all persimmons sample slices during storage time (Table 2). In persimmon fruit, levels of ascorbic acid are considerably greater (10 times) in cultivars that are non-astringent than in cultivars that are astringent [26]. Our results on *Rojo Brillante* showed that ascorbic acid content decreased in all treatments from cut to the 9th day of storage. Significant differences were shown between treatments on 6th day of storage when CTR+MAP samples had lower mean values (0.1 μ mol g⁻¹ fw) than other treatments. Malic acid content decreased in all treatments until the 9th day of storage registering significant differences of about 11%.

Veberic et al., [27] showed that total organic acid content could change by different cultivars. The authors reported different a level ranging from 681 ± 26.4 mg kg $^{-1}$ in cultivar 'Jiro' to more than twice in cultivars 'Triumph' and 'Tipo', which on average contained 1439 mg kg $^{-1}$, of organic acids. Amongt the

individual organic acids, the highest content of malic acid ($1044\pm43.2~{\rm mg~kg^{-1}~FW}$) was measured in cultivar 'Triumph', and the lowest ($401\pm16.7~{\rm mg~kg^{-1}~FW}$) in cultivar 'Jiro'.

Citric acid content decreased in all times and treatments. CTR+MAP and CTR PASSIVE treatments showed significant differences on 9th day reporting lower values than other treatments. Similar results shown that citric acid decreases during ripeness while malic acid increases [28].

The same citric acid trend occurred on succinic acid content showing significant differences immediately after 3 days of storage. Those results showed that EC+MAP and CTR PASSIVE treatment had the highest mean values in terms of succinic acid content. In another work on persimmon fruit succinic acid increased after 2 days of storage at 13 °C and 27 °C [29]. In breba fig fruit, malic acid and citric acid were higher in *Opuntia ficus-indica* mucilage-coated sample than in untreated samples used as control, analyzed at commercial harvest time [30]. The addition of ascorbic and citric acids in edible coating solutions may have affected their content in persimmon slices, while titratable acidity content did not show significant differences between treatments ($P \le 0.05$).

Table 2 Organic acid (ascorbic, malic, citric and succinic acids) in minimally processed persimmon fruit (*Diospyros kaki* L.), cv *Rojo Brillante* immediately after harvest (T0) and after 3, 6 and 9 days of storage

Time of storage	Organic acid (µmol.g ⁻¹ fw)	CTR PASSIVE	EC + MAP	CTR + MAP	EC PASSIVE
TO	Ascorbic acid	0.7 ns	0.7	0.7	0.7
	Malic acid	176.3 ns	176.3	176.3	176.3
	Citric acid	6.3 ns	6.3	6.3	6.3
	Succinic acid	5.9 ns	5.9	5.9	5.9
	TA (% malic acid)	0.1 ns	0.1	0.1	0.1
	Ascorbic acid	0.4 ns	0.5	0.3	0.5
T3	Malic acid	159.3 ns	160.3	154.4	156.7
	Citric acid	4.6 ns	5.3	4.4	4.6
	Succinic acid	5.2a	5.1a	4.5a	3.6b
	TA (% malic acid)	0.1 ns	0.1	0.1	0.1
	Ascorbic acid	0.3a	0.4a	0.1b	0.3a
T6	Malic acid	158.3 ns	158.4	155.2	153.1
	Citric acid	4.8a	5.0a	3.3b	4.1a
	Succinic acid	5.1a	4.5a	4.1ab	3.1b
	TA (% malic acid)	0.1 ns	0.1	0.1	0.1
	Ascorbic acid	0.1 ns	0.3	0.1	0.3
Т9	Malic acid	136.3b	150.2a	151.7a	135.3b
	Citric acid	3.5b	5.2a	3.5b	4.0b
	Succinic acid	4.4a	4.6a	3.1b	2.9b
	TA (% malic acid)	0.1 ns	0.1	0.1	0.1

At each sampling date, different letters indicate substantial changes between treatments ns non-significant

 $P \le 0.05$ was used in the Tukey's significant test

Firmness and weight loss

Persimmon fruit suffered rapid softening, [31, 32] while the use of 1-methylcyclopropene exhibited gradual softening during storage at 20 °C [25]. In our samples, firmness showed a decrease of mean values in all sample times at 5 °C. Significant differences occurred between treatments after 3, 6 and 9 days when CTR PASSIVE and CTR + MAP showed lower mean values if compared to EC + MAP and EC PASSIVE (Fig. 1).

The flesh deterioration appeared to be associated with the increased activity of PPO (Fig. 4). Others authors [33] have shown that an increase of PPO activity in

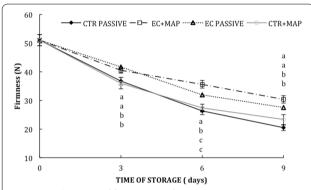


Fig. 1 Development of firmness (N) of treated (CTR PASSIVE, EC PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cut of persimmon (*Diospyros kaki* L.), CV *Rojo Brillante* just after cut (0) and at 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test. Data are provided as a mean standard error (n=3) average

persimmon fruit *cv. Fuyu* bruised. The persimmon slices treated with EC+MAP reported the best results in terms of firmness. Probably, calcium content in EC has protected and maintained fruit quality by enhancing antioxidant capacity, avoiding softening, and preventing deterioration during shelf life [13, 34]. Indeed, the addition of calcium on fresh-cut fruit stability improved the stiffness of the middle lamella and cell walls.

Persimmon slices treatments reported an increase in mean values in terms of weight loss, but CTR PASSIVE samples showed a weight loss of 3.2% and 3.0% after 6 and 9 days, respectively (Fig. 2).

In EC+MAP, EC PASSIVE and CTR+MAP there were no significant differences after 3, 6 and 9 days (Fig. 2).

In another study, no significant differences were found in firmness of *Fuyu* persimmons coated with gelatin-based frog skin oil stored for up to 9 days at 25 °C [35] while on *Rojo Brillante* persimmons, starch–gellan coatings formulated with and without thyme essential oil maintained higher fruit firmness than uncoated fruit for 14 days of storage at 25 °C [36].

Browning index and PPO activity

Color is an analytical parameter that has a significant impact on consumer approval, since it is the first aspect he considers [37, 38]. From the data analysis, treatments had a considerable influence on fruit quality.

The mean values of browning index in treated and untreated persimmon slices increased during storage time (Fig. 3).

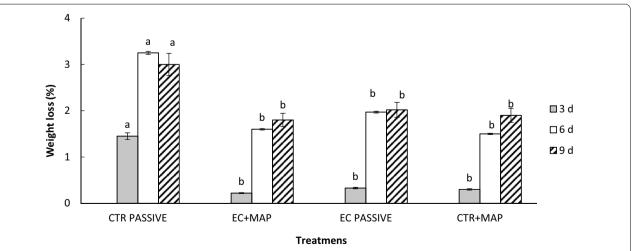


Fig. 2 Development of weight loss (%) of treated (CTR PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cut of persimmon (Diospyros kaki L.), cv *Rojo Brillante* just after cut (0) and at 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test. Data were provided as a mean standard error (n = 3) average

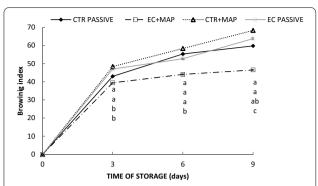


Fig. 3 Development of browning index of treated (CTR PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cut persimmon (*Diospyros kaki* L.), cv *Rojo Brillante* just after cut (0) and after 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test

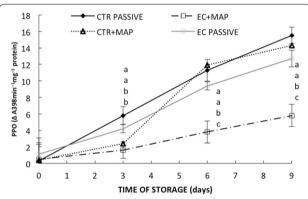


Fig. 4 Development of PPO of treated (CTR PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cuts of persimmon (*Diospyros kaki* L.), cv *Rojo Brillante* just after cut (0) and at 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test. Data are provided as a mean standard error (n = 3) average

On 3rd day of storage EC+MAP and CTR PASSIVE showed significant differences if compared to other treatments. EC+MAP persimmon slices showed lower mean values than other treatments from 3 to 9 days of storage, registering significant differences between treatments only after 6 and 9 days (Fig. 3). In another work, the use of CaCl₂ reduced enzymatic browning on fresh-cut persimmon, having higher hue and lower ΔE values (change color) than the control samples, but its effectiveness was lower than ascorbic and citric acid, with lower hue values [39].

The same treatment showed lower mean values than other treatments in terms of PPO activity from on 3rd to 9th day of storage at 5 °C (Fig. 4).

No differences occurred on 3rd day of storage between EC+MAP and CTR+MAP treatments while, after 3 days, EC+MAP treatment showed the lowest values (Fig. 4). CTR Passive and EC passive reported an increase in PPO activity while CTR+MAP remained stable only on 3rd day of storage and then increased during storage. In another study on *Fuyu* persimmon bruised fruit, the increment of PPO activity seemed to be associated with flesh deterioration [33] while ascorbic and citric acid and cysteine on fresh-cut *Rojo Brillante* persimmon, proved to be the most effective antioxidants to control enzymatic browning [39].

Carotenoids content

Persimmons fruits are a good source of carotenoids, mostly found in skin and flesh, which are responsible for the color of the fruits and whose amount increases as the fruit ripens [40, 41]. Zhou et al. [42], showed that β-cryptoxanthin was the most abundant carotenoid among all individual components in both peel and flesh. The same authors reported that zeaxanthin was also the most abundant in all persimmons fleshes besides β-cryptoxanthin. In our work, we evaluated α-cryptoxanthin, β -cryptoxanthin, α -carotene, β -carotene and total carotenoids during storage. As reported by Niikawa et al., [43], β-cryptoxanthin accumulates immediately after skin and flesh color changes. After cutting, the mean values of α -cryptoxanthin, β -cryptoxanthin, α -carotene, β -carotene and total carotenoids showed a decrease in all time and all treatments. EC+MAP and EC PASSIVE treatments showed higher mean values in terms of α -cryptoxanthin content than CTR PASSIVE and CTR+MAP during cold storage. EC+MAP persimmon slices showed higher mean values in terms of β -cryptoxanthin content than other treatments during storage time.

The same significant differences occurred on EC PAS-SIVE if compared to other treatments, in terms of α -carotene and β -carotene from cut to 9th day (Table 3). Significant differences occurred on total carotenoids showing the higher mean values of EC+MAP compared to other treatments while in other works carotenoids content of fresh-cut persimmons (Sanchis et al., 2015) and kiwifruit [44] were not affected by antioxidant treatment, nor by storage at 5 °C.

In other works, the use of low-molecular-weight chitosan (LC)–(-)-epigallocatechin-3-gallate (EGCG) delayed the degradation due to the β -carotene selective

Table 3 Evolution of carotenoids (α -cryptoxanthin β -cryptoxanthin α -carotene β -carotene total carotenoids), in minimally processed persimmon fruit (*Diospyros kaki* L.), cv *Rojo Brillante* immediately after harvest (T0) and at 3, 6 and 9 days of storage

Time of storage	Carotenoids µg/100 g	CTR PASSIVE	EC + MAP	CTR + MAP	EC PASSIVE
ТО	α-Cryptoxanthin	55.3	55.3	55.3	55.3
	eta-Cryptoxanthin	160.3	160.3	160.3	160.3
	lpha-Carotene	18.5	18.5	18.5	18.5
	eta-Carotene	112.4	112.4	112.4	112.4
	Total	342.7	342.7	342.7	342.7
T3	lpha-Cryptoxanthin	48.5ab	50.3a	47.1b	49.7a
	eta-Cryptoxanthin	128.4c	148.4a	135.3b	146.3a
	lpha-Carotene	10.8c	12.6ab	11.9b	13.8a
	eta-Carotene	78.7c	80.8b	77.3c	85.7a
	Total	287.3a	297.5a	179.8b	288.3a
	lpha-Cryptoxanthin	44.6b	47.4a	45.3b	48.3a
	$oldsymbol{eta}$ -Cryptoxanthin	126.7c	144.3a	123.3c	139.3b
T6	lpha-Carotene	9.3b	10.2ab	9.1b	11.1a
	eta-Carotene	76.9a	77.4b	65.3c	79.3a
	Total	260.1c	283.3a	160.3d	270.7b
	lpha-Cryptoxanthin	41.1b	46.3a	42.2b	45.4a
	eta-Cryptoxanthin	109.7c	141.0a	110.4c	122.2b
Т9	lpha-Carotene	8.6a	5.3c	7.3b	8.1a
	eta-Carotene	70.1a	50.2b	55.3b	71.3a
	Total	210.2b	266.3a	154.3c	198.2b

At each sampling date, different letters indicate substantial changes between treatments

permeability of chitosan coating. The high β -cryptoxanthin 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 commercial ripe provided between 5 and 8% of the RDA in a 100 g serving. This contribution was larger than those reported in literature for other persimmon cultivars [26].

Sensory quality

After 3 days of storage, significant differences occurred in terms of flavor and overall evaluation among the treatments (Fig. 5). Furthermore, EC+MAP fruits recorded the highest value with an average score of 8; CTR+MAP and CTR PASSIVE fruit had a score of 6.

On 6th day of storage, significant differences were found in terms of consistency, smell of fruit and flavor. EC-treated fruits, both MAP and PASSIVE, was in a range of 6 and 5 for what concerns the overall evaluation (OVE), CTR PASSIVE and CTR+MAP, instead, recorded a value of 3.4 and 4, respectively, for the same descriptor OVE (Fig. 6).

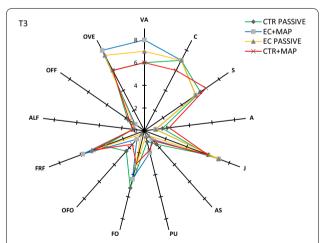


Fig. 5 Sensory analysis of treated (EC + MAP and EC passive) and untreated (CTR Passive and CTR + MAP) fresh-cut of persimmon on 3rd day of storage at 5 °C. Legend: visual appearance (VA); compactness (C); sweetness (S); acidity (A); juiciness (J); astringent (AS); pungent (PU); fruit odor (FO); floury (FAR); off-odor (OFO); fruit flavor (FRF), alcoholic flavor (ALF), off-flavor (OFF) and overall evaluation (OVE). Data correspond to the means ± standard deviations of three replicates

 $P \le 0.05$ was used in the Tukey's significant test. Data are provided as a mean. (n = 3) average

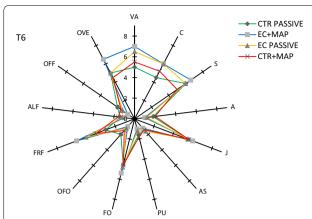


Fig. 6 Sensory analysis of treated (EC + MAP and EC passive) and untreated (CTR Passive and CTR + MAP) fresh-cut of persimmon on 6th day of storage at 5 °C. Legend: visual appearance (VA); compactness (C); sweetness (S); acidity (A); juiciness (J); astringent (AS); pungent (PU); fruit odor (FO); floury (FAR); Off-odor (OFO); fruit flavor (FRF), alcoholic flavor (ALF), off-flavor (OFF) and overall evaluation (OVE). Data correspond to the means ± standard deviations of three replicates

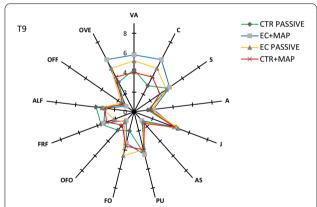


Fig. 7 Sensory analysis of treated (EC + MAP and EC passive) and untreated (CTR Passive and CTR + MAP) fresh-cut of persimmon on 9th day of storage at 5 °C. Legend: visual appearance (VA); compactness (C); sweetness (S); acidity (A); juiciness (J); astringent (AS); pungent (PU); fruit odor (FO); floury (FAR); off-odor (OFO); fruit flavor (FRF), alcoholic flavor (ALF), off-flavor (OFF) and overall evaluation (OVE). Data correspond to the means ± standard deviations of three replicates

On 9th day of storage, visual appearance, compactness, sweetness and overall evaluation recorded the highest values in EC+MAP, followed by EC PASSIVE treated sample (Fig. 7). It was possible to state that EC treatments significantly prevent the loss of firmness and the organoleptic decay of persimmon fruits. The use of edible coating on fresh-cut persimmon fruit did not

affect off-flavor during cold storage time. Similar results showed on fresh-cut persimmon fruit treated with honey, prevented off-aroma development and delayed jelling. However, the softness and exuding juice of the fresh-cut persimmon cubes increased with time, with the increase in both parameters being significantly suppressed by honey solution dips [45].

CO₂ and O₂ inside packaging

The rate of CO₂ production exhibited a typical climacteric pattern of respiration during ripening of persimmon fruit at 20 °C. As reported by Sanchis [46], the combination of high O2 (21 kPa) and elevated CO2 (10 or 20 kPa) did not prevent enzymatic browning and softening of fresh-cut Rojo Brillante persimmons, and high CO₂ concentrations induced flesh browning on tissues. To prevent browning phenomena, antibrowning agents and MAP proved to be the most effective combination to prevent enzymatic browning and maintain visual quality above the limit of marketability for 9 days at 5 °C. In our work, an increase was observed from day 3 to day 6 of storage in CTR MAP and EC MAP. The EC+MAP treatment greatly inhibited CO₂ production in persimmon fruit during the first 3 days. On 6th and 9th day of storage CO₂ content inside packaging increased of 30% and 43.3%, respectively. Significant differences between CTR + MAP and EC + MAP occurred only at 12th day of storage (Fig. 8). No significant differences were detected between CTR passive and EC passive treatments in all time of storage. In fresh-cut peach stored under passive atmosphere, with or without chemical treatment, the shelf life was extended by up to 7 days [47].

With regard to oxygen (O₂) content inside packaging, CTR passive and EC passive showed a sharp decrease

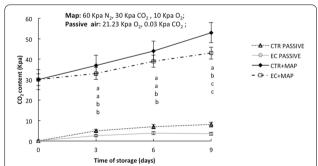


Fig. 8 Development of carbon dioxide (CO₂)content of treated (CTR PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cut of persimmon (*Diospyros kaki* L.), cv *Rojo Brillante* just after cut (0) and after 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test. Data are provided as a mean S.E. (n = 3) average

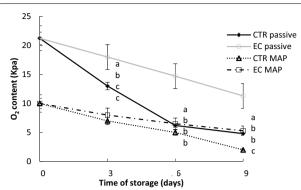


Fig. 9 Development of oxygen (O₂) content of treated (CTR PASSIVE, EC PASSIVE, EC + MAP and CTR + MAP) fresh-cut of persimmon (*Diospyros kaki* L.), cv *Rojo Brillante* just after cut (0) and after 3, 6, 9 days of storage at 5 °C. At each sampling date, different letters indicate substantial changes between treatments. $P \le 0.05$ was used in the Tukey's significant test. Data are provided as a mean S.E. (n = 3) average

during storage time, while other treatments reported a slightly decrease in mean values. Significant differences occurred between CTR and EC passive treatments during the storage period and no significant differences were recorded between EC MAP and CTR MAP treatments (Fig. 9). The effect of the edible coating to reduce respiration rate of persimmon slices was only observed in samples packed under active MAP. EC has proved to be a good way to decrease the respiration rate of fruits, by creating a semipermeable layer that minimizes gas exchange. Indeed, Benitez et al., [48] reported the effect of Aloe ν . edible coatings on reducing of CO_2 production in kiwi fresh-cut fruit. In other works, the effects of Aloe ν . coating in reducing respiration rate on table grapes [37] and on cherry fruit during storage at 1 °C and at 20 °C [49] were shown.

Microbiological analysis

Rojo Brillante persimmon is considered a perishable fruit, due to its susceptibility to microbial spoilage since it exhibits a pH around 6 [22]. Therefore, a microbiological analysis is an important factor to consider for the preservation of these fruits during handling or processing. The microbial loads detected on the different fruit samples collected during the experimentation are reported in Tables 4, 5. The results of viable counts performed on A. vera coating did not evidence the presence of any of the microbial groups objects of investigation (for this reason, these results are not

Table 4 Evolution of microbial loads of ready-to-eat persimmon fruits samples (CTR + MAP, EC + MAP) during cold storage (9 days)

Microorganism	CTR+M/	AP			EC+MAP			
	0 d	3 d	6 d	9 d	0 d	3 d	6 d	9 d
TMM	< 1 ^a	< 1 ^a	4.8 ± 0.2 ^b	6.2 ± 0.2 ^b	< 1 ^a	< 1 ^a	< 1 ^a	< 1a
Pseudomonads	< 1 ^a	< 1 ^a	4.8 ± 0.2^{b}	6.3 ± 0.3^{b}	< 1 ^a	< 1a	< 1 ^a	< 1a
Enterobacteriaceae	< 1 a	< 1a	2.7 ± 0.1^{b}	2.8 ± 0.3^{b}	< 1a	< 1a	< 1 a	< 1a
Yeasts	< 1a	< 1a	3.3 ± 0.3^{b}	4.3 ± 0.2^{b}	< 1a	< 1a	< 1a	< 1a
Molds	< 1 a	< 1a	3.4 ± 0.2^{b}	4.2 ± 0.1^{b}	< 1a	< 1 a	< 1 a	< 1 ^a

 $^{^{\}rm a}$ Units are log cfu/g. Results indicate mean values \pm S.D. of two plate counts

 $\textit{CTR} \ control\ production, \textit{EC}\ experimental\ production, \textit{TMM}\ total\ mesophilic\ microorganisms$

Table 5 Evolution of microbial loads of ready-to-eat persimmon fruits samples (CTR PASSIVE, EC PASSIVE) during cold storage (9 days)

Microorganism	CTR PASS	IVE			EC PASSIVE	VE		
	0 d	3 d	6 d	9 d	0 d	3 d	6 d	9 d
TMM	< 1 ^a	< 1 ^a	3.8 ± 0.2 ^b	6.8 ± 0.2 ^b	< 1 ^a	< 1 ^a	< 1 ^a	< 1a
Pseudomonads	< 1a	< 1a	4.2 ± 0.2^{b}	6.6 ± 0.3^{b}	< 1a	< 1a	< 1a	< 1a
Enterobacteriaceae	< 1 a	< 1a	2.1 ± 0.1^{b}	$3.3 \pm 0.3^{\circ}$	< 1 ^a	< 1a	< 1 ^a	< 1a
Yeasts	< 1a	< 1 ^a	3.6 ± 0.3^{b}	$5.3 \pm 0.2^{\circ}$	< 1a	< 1 ^a	< 1 a	< 1a
Molds	< 1a	< 1a	3.8 ± 0.2^{b}	$5.2 \pm 0.1^{\circ}$	< 1a	< 1a	< 1a	< 1a

 $^{^{\}rm a}$ Units are log cfu/g. Results indicate mean values \pm S.D. of two plate counts

^b Data within a line followed by the same letter for the CTR and EC at the same day are not significantly different according to Tukey's test

^b Data within a line followed by the same letter for the CTR and EC at the same day are not significantly different according to Tukey's test *CTR* control production, *EC* experimental production, *TMM* total mesophilic microorganisms

reported in Table 4). According to Tukey's test, statistically significant differences among treatments appeared after 6 d of storage when CTR+MAP (Table 4) and CTR PASSIVE (Table 5) samples showed levels of TMM, TPC, yeasts and molds higher than 10³ CFU/g, while these microbial groups were below the detection limit in EC+MAP (Table 4) and EC PASSIVE (Table 5) fruits at each storage time. The levels of members of *Enterobacteriaceae* family, that might include potential pathogenic microorganisms [50], were below the detection limit for CTR+MAP and CTR PAS-SIVE treatments after 3 days of storage, but they increased at around 10³ Log CFU/g at 9 d. On the other hand, no colonies of these bacteria were detected in EC+MAP and EC PASSIVE. These results confirmed previous investigations [49, 51] which evaluated the effects of A. vera coating in apple slices and sweet cherry and showed a reduction of mesophilic aerobic bacteria, as well as yeast and mold counts. The absence of microorganisms in EC fruit samples confirmed the antibacterial activity of *A. vera* [52] even after edible coating application.

Conclusion

The application of *Aloe vera* coating formulated with antibrowning agents and storage with MAP significantly extended the shelf life of *Rojo Brillante* persimmon slices during 9 days of storage at 5 °C. The EC+MAP proved to be the most effective treatment to maintain the total carotenoids, glucose and CO₂ inside packaging reducing the PPO activity and the flesh browning of persimmon slices. EC+MAP slices were evaluated as very good at the end of the 9-day storage period, while the fruit flavor fell within the limit of acceptability. Therefore, the *Aloe* vera coating represents a hurdle to the microbial spoilage of persimmon, and it also allows to reduce microbial risk associated to the presence of potential pathogenic microorganisms. EC+MAP treatment stunted the growth of TMM, pseudomonads, Enterobacteriaceae, yeasts and molds. Furthermore, EC+MAP treatment reduced water loss, and maintained fruit firmness, suggesting that this treatment was an effectively integrated strategy in extending the storage life of the fresh-cut of persimmon.

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Author contributions

Conceptualization, GS, FS, VF and AA; methodology, GS, VF, RG and AA; software, GS, AA, VF and RP; validation, AA and GS and VF; formal analysis, GS, RP, RG, AA, VF. and FS; investigation, GS, AA, RP and RG; resources, AG and GS; data curation, G.S., A.A. A.G. and VF; writing—original draft preparation, AA, VF, RG, AG and GS; writing—review and editing, AA., GS and PI; visualization, AA, VF, RG and GS; supervision, VF, GS and PI; funding acquisition, AG and GS. All authors reviewed the manuscript. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

G.S. is an associate editor of *Chemical and Biological Technologies in Agriculture*. The rest of the authors have no conflicts of interest.

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