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Active label-based packaging to extend the shelf-life of "Calanda" peach fruit: Changes in fruit quality and enzymatic activity

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ABSTRACT

A new active packaging, consisting of a label with cinnamon essential oil incorporated and attached to plastic packaging, was used to extend the shelf-life of late-maturing peach fruit. After 12 days of storage at room temperature, the percentage of infected fruit in the active label packaging was 13% vs. 86% in the non-active packaging. Significant differences were obtained for weight loss (3.4% less at 12 days of storage) and firmness (more than 15.9 N at 12 days) during storage. The influence of the active packaging on the *in vivo* activity of lipoxygenase (LOX), polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and of malondialdehyde (MDA) content as an indicator of lipid oxidation, was studied. The active agent, cinnamon essential oil, also reduced *in vitro* activity of LOX. Sensory analysis of the peaches was performed over the storage time. Most of positive descriptors were not significantly different from the optimum quality level (day 0) for peaches stored in the active package after 12 days at room temperature.

1. Introduction

Technologies used to extend shelf-life of foodstuffs include active packaging. This consists of incorporating active agents into packaging, which interact with food through various mechanisms such as eliminating undesirable compounds or adding beneficial compounds to the product (Vermeiren et al., 1999). Several studies have shown the potential benefit of using essential oils as active agents in packaging materials, maintaining the initial characteristics of the food, protecting it from microbial spoilage and therefore extending shelf-life (Burt, 2004). Among these, Neri et al. (2007) have reported antifungal activity of several plant volatile compounds against *Monilinia laxa,* which causes brown rot in stonefruit.

The incorporation of essential oils into a package has usually been made by direct incorporation into the plastic polymer, or by using a coating on the polymer (Lopez et al., 2007; Gutiérrez et al., 2009; Rodriguez-Lafuente et al., 2010). However, the use of active labels has not been extensively used to date (Winther and Nielsen, 2006). Labels are very versatile and also easily adapted to an industrial scale and can be added just before packaging the food, thereby maximizing their functionality. This paper explores the use of auto-adhesive labels containing cinnamon essential oil as the active agent to improve the shelf-life of "Calanda" peach fruit (*Prunus persica* Sieb and Zucc).

"Calanda" peach is a highly added value variety of peach from the Spanish Origin Denomination "Melocotón de Calanda". This peach is a clingstone non-melting variety with yellow flesh. Its shelf-life is only from 3 to 5 days at room temperature (20–22 °C), mainly due to rapid ripening once harvested and further microbial spoilage, limiting its market availability to a small geographical area. Previous studies have shown the susceptibility of the fruit to chilling injury, and therefore, low temperatures such as 2–8 °C are not effective for extending shelf-life. However, some chilling-related disorders and fruit senescence could be avoided or delayed by storing fruit at 0 °C (Crisosto, 2002; Fernández et al., 2009).

The deterioration of peaches occurs in three ways: physical, chemical and biological. The physical and chemical damage (Hariyadi and Parkin, 1991; Wang et al., 2004) can be controlled with a proper handling and processing during the harvest. The biological damage is from action of parasites, enzymatic effects (Khan and Singh, 2007) and microorganisms such as *Monilinia fructicola* and *Penicillium expansum,* causing the change of color to brown (brown rot) or the appearance of blue mold, or gray mold, caused by *Botrytis cinerea* (Karabulut and Baykal, 2002; Karabulut et al., 2002; Guijarro et al., 2007). The damage caused by the Mediterranean fly is usually avoided by bagging the fruit peaches individually while

Abbreviations: CAS, chemical abstract service; CAT, catalase; EDTA, ethylenediaminetetraacetic acid; EOs, essential oils; LOX, lipoxygenase; LSD, least significant difference; MDA, malondialdehyde; NBT, nitroblue tetrazolium; PET, polyethylene terephthalate; POD, peroxidise; PP, polypropylene; PPO, polyphenol oxidase; SOD, superoxide dismutase; SSC, soluble solids content; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

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still on the tree (Fernández et al., 2009). The production and elimination of reactive oxygen species is strongly linked to the activity of the enzymes. Lipoxygenase (LOX) catalyzes the peroxidation of unsaturated fatty acids (with a *cis*,*cis*-1,4-pentadiene system) from the cellular wall (Baysal and Demirdöven, 2007). As a consequence of this cellular damage, some existing compounds, such as phenols, are oxidized to ortho-quinones with the help of polyphenol oxidase (PPO), resulting in the typical brown coloring of tissues (Reyes and Luh, 1960; He et al., 2008). The brownish color might also appear as a result of chemical oxidation with the production of MDA (Shen et al., 2006).

The main aim of this work was to demonstrate that new autoadhesive active labels incorporated into the plastic package can be successfully used to extend the shelf-life of "Calanda" peaches at room temperature. Decay evaluation and some selected physicochemical parameters (weight loss, soluble solids content, firmness and color) of the peaches were controlled. The activities of five enzymes including LOX, and MDA content, were measured throughout the storage time. Finally, sensory studies were carried out to evaluate the sensory characteristics of the fruit in the proposed active packaging in order to detect whether the active label modified fruit flavor.

2. Materials and methods

2.1. Fruit samples and essential oils

One clone of *Prunus persica* Sieb & Zucc (Jesca clone) was studied as the most representative of this variety. The fruit were collected at the optimum harvested stage from a horticultural farm (Calanda, Spain) and transported to the laboratory within the same day. They were selected according to apparence/visual uniformity. Only peaches free from damage were selected and were randomized before testing to avoid bias.

Experiments were carried out during October of 2007, 2008 and 2009. Table 1 summarizes the experiments and the year in which they were carried out. Cinnamon essential oil (*Cinnamomum zeylanicum*) containing *trans*-cinnamaldehyde (75–85%), eugenol (5–10%) and *d*-Limonene (0–5%) together with other minor compounds such as *p*-cymene and linalool, was used as the active agent. Cinnamon Essential Oil (Cinnamon EO hereafter) was supplied by Argolide Química (Barcelona, Spain).

2.2. Chemicals

All the standards and reagents were supplied by Sigma–Aldrich (Madrid, Spain). These included lipoxygenase (LOX, Chemical Abstract Service (CAS) Registry No. 9029-60-1), polyphenol oxidase (PPO, CAS 9002-10-2), peroxidase (POD, CAS 9003-99-0), superoxide dismutase (SOD, CAS 9054-89-1), catalase (CAT, CAS 9001-05-2), malondialdehyde (MDA, CAS 100683-54-3), polyvinyl polypyrrolidone (PVPP, CAS 9003-39-8), Trizma® hydrochloride buffer solution (Tris–HCL, pH 9.0), sodium phosphate (CAS 7601-54-9), sodium hydroxide 1 M solution (as pH adjustment, CAS 1310-73-2), linoleic acid (CAS 60-33-3), Tween 80 (CAS 9005-65-6), catechol (CAS 120-80-9), guaiacol (CAS 90-05-1), hydrogen peroxide (CAS 7722-84-1), riboflavin (CAS 83-88-5), nitroblue tetrazolium (NBT, CAS 298-83-9), ethylenediaminetetraacetic acid disodium salt (EDTA, CAS 139-33-3), thiobarbituric acid (TBA, CAS 504-17-6) and trichloroacetic acid (TCA, CAS 650-51-1).

Table 1
Recap of the experimental tests carried out with "Calanda" peach. Harvesting dates are shown in brackets. "×" means that the test was conducted.

Experimental	Year		
	2007 (10th October)	2008 (8th October)	2009 (6th October)
Antifungal assay	×	×	×
Weight loss	×	×	×
Color	×	×	×
Firmness	–	–	×
Soluble solids content	–	×	–
Enzymatic analysis	–	×	–
Sensory analysis	×	–	–

2.3. Active packaging manufacture

Two prototypes of active packaging were studied, both containing cinnamon EO as the active agent. The first prototype (active tray, hereafter) consisted of a macro-perforated tray of polyethylene terephthalate (PET) with a known concentration of active ingredient of 0.062 g m^{-2}. Briefly, the active material was prepared by coating the material with a formulation containing cinnamon EO. The dimensions of the active PET trays were 18 cm × 18 cm × 7 cm (2268 cm^3 volume), however only the bottom of the tray was coated with the active formulation (324 cm^2 of active surface). The size of this active tray allowed storage of up to 4 peach fruit (990 g). Thus, the fruit mass per active tray surface was 3.1 g cm^{-2}.

The second prototype (active label, hereafter) consisted of a self-adhesive transparent polypropylene (PP) active label (100 cm^2 surface area) which was stuck onto the macro-perforated tray of PET (18 cm × 18 cm × 7 cm) on the top lid, as shown in Fig. 1. The active labels were obtained by coating the label with a formulation containing cinnamon EO. The concentration of cinnamon EO in the final packaging was in this case 0.54 g m^{-2}, and the fruit mass per active tray surface ratio was 9.9 g cm^{-2}.

Both the active trays and the active labels were prepared 24 h prior the tests and supplied by the company ARTIBAL S. A. (Sabiñánigo, Spain). This methodology is protected by the European Patent EP1657181 and held by the company ARTIBAL S.A. The active labels were stuck onto the PET trays 30 min prior to filling the trays with the peaches.

2.4. Antifungal assays

Peaches were stored at two temperatures, room temperature (20–25 °C) and low temperature (0–0.5 °C) for both the active trays and the active labels. Controls (non-active macro-perforated trays of PET) were also tested for each set of samples. Each treatment was replicated three times and the experiment was conducted in triplicate (Table 1).

Fungal infection was visually evaluated atfter 4, 8, 12, 16 and 20 days of storage. Fruit with visible mold were considered as "infected", and the degree of infection was not rated. The visible decay on the fruit for each packaging was expressed as the percentage of infected peaches (n = 9).

2.5. Physico-chemical analysis

Some physico-chemical parameters (weight loss, color, firmness and soluble solids content) were monitored before treatment and during storage to evaluate the effect of the active packaging on the peach fruit. The loss of water was gravimetrically assessed with time. Active and control trays containing the peaches were weighed at specific time intervals (0, 4, 8, 12, 16 and 20 days of storage). Cumulative weight losses were expressed as percentage loss of the initial weight.

Color of the peaches was measured with a CR-400 chroma meter, illuminant C (Konika Minolta, Tokyo, Japan). Four points at the equatorial perimeter of the fruit skin were measured on three peaches from each tray and from each packaging treatment.

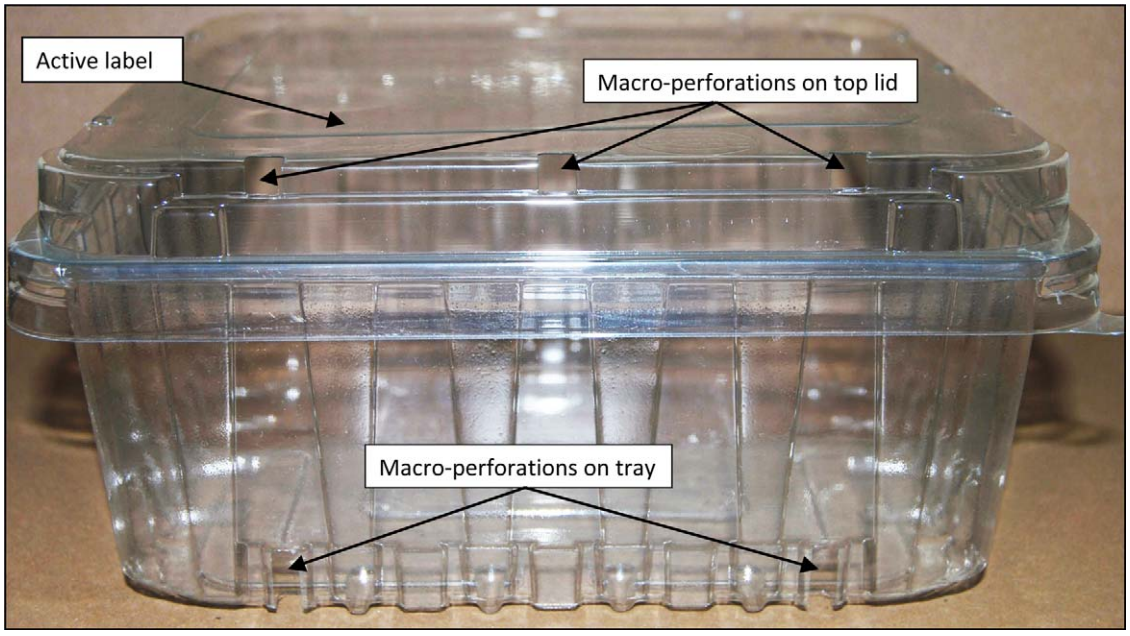

Fig. 1. Active label packaging. The trays of PET included 20 macro-perforations, 8 at the bottom of the tray (19 mm × 5 mm) and 12 at the top lid (6 mm × 9 mm).

Each packaging treatment was evaluated in triplicate, thus a total number of 36 measurements were taken for each packaging treatment. Color changes were quantified in the CIELAB color space (L*, a, b). L* refers to the lightness, chroma represents color saturation and hue angle is defined as a color wheel (McGuire, 1992, Equations 1 and 2). Although L*, C* and h° values are used to measure the absolute color, it is more interesting to determine the color differences. For this reason, the CIE 1976 color difference (Equation 3) proposed by the International Commission on Illumination in 1976 was used. ΔE represents the color difference and it is the Euclidean distance in the color space, and ΔL^*, Δa^* and Δb^* are the lightness difference and a* and b* differences for two independent measures (Meléndez-Martínez et al., 2005).

Four fruit per each combination of factors (package and storage period) were analyzed for firmness. It was measured on two opposite sides of each fruit at the equatorial zone with a Fruit Firmness Tester FT-327 (TR snc, Italy), fitted with an 8 mm plunger tip. Soluble solids content in peach juice was determined using the Brix Index (°Bx). The pulp of the peaches (40 g) was crushed and homogenized, and then an aliquot of 2 mL was directly measured with a digital refractometer MTD 045nD (Three-in-one Enterprises, Taipei, Taiwan). Ten fruit per each combination of factors (package and storage period) were used for soluble solids content analysis.

2.6. In vitro lipoxygenase analysis

The procedure for the LOX assay was performed according to Naidu (1995) with minor changes. *In vitro* enzymatic lipid peroxidation was measured by spectrophotometry following the increment of absorbance at 234 nm based on lipid hydroperoxides formation. The reaction medium in the cuvette was a buffer solution containing 100 mM sodium phosphate, 50 μL 10 mM linoleic acid, 50 μL 150 U mL^{-1} lipoxygenase enzyme, and 0, 50 and 100 μL cinnamon EO (3.05 mL final volume). Blanks were prepared with the buffer solution and 0, 50 and 100 μL of cinnamon EO up to a total volume of 3.05 mL. A UV-1700 Pharmasec Spectrophotometer (Shimazdu, Japan) was used for the absorbance measurements. LOX was preincubated for 5 min with different concentrations of cinnamon EO prior to initiation of the reaction with the linoleic acid.

2.7. In vivo enzymatic analysis

All the enzymatic analyses were performed at 0, 4, 8, 12 and 16 days. Three trays from each packaging treatment were taken to provide three treatment replicates. In each case, three peaches from each tray were peeled and their skin was homogenized to obtain a representative sample of each packaging treatment. The pure enzymes were used as reference standards in each particular case at each sampling point as described below. Then, the control and active samples were analyzed each day, to facilitate the comparison of the values from control and active packaging treatments.

The entire procedure for the extraction of the enzymes and determination of MDA was performed according to the method of Wang et al. (2004). Peach skin instead of flesh was sampled for the enzyme analysis, since several authors have stated that the differences in the use of skin and pulp are not significant, and even in some cases like "Fuji" apples, the skin presents better results (Echeverria et al., 2004). 10 g of peach skin from three or four fruit for each treatment were collected, accurately weighed, ground in a mortar, blended with a mixer, homogenized and extracted with 25 mL of the appropriate cold buffer (see below), containing 0.5 g of PVPP. The mixture was vigorously shaken and centrifuged at 20,000 × *g* for 60 min using a Centromix 5-549 (Selecta, Spain), the supernatant liquid was collected, filtered through 40 μm-pore size filter paper and analyzed immediately. LOX was extracted with a 100 mM Tris–HCl buffer solution (pH 8.0), a 100 mM sodium phosphate buffer (pH 6.4) was used to extract SOD, POD, PPO and MDA, and finally, CAT was extracted with a 50 mM sodium phosphate buffer solution (pH 7.0).

The methods to measure the activity of the enzymes were adapted (with slight modifications in some cases) from the following: LOX activity according to the method of Axelrod et al. (1981) and Perez et al. (1999), PPO and POD activity and MDA content according to the method of Jiang et al. (2002), SOD activity was measured according to the method of Giannopolitis and Ries (1977) with the modifications of Wang et al. (2004), and CAT activity was

obtained according to the method of Beers and Sizer (1952) with the modifications of Wang et al. (2004). Measurements of absorbance were carried out by UV–vis spectrometry, using a UV-1700 Pharmasec Spectrophotometer. The volume of the cuvette was 4.2 mL.

The activity of LOX was measured on the basis of the conjugated dienes at 234 nm and 30 °C, a buffer solution (2.85 mL) containing 100 mM sodium phosphate (pH 6.0), 50 μL of 10 mM linoleic acid and 0.1 mL of the extract (total volume of 3.0 mL). 10 mM aqueous solution of linoleic acid was stabilized with Tween 80. Blanks were prepared with 2.95 mL of the buffer solution and 50 μL of 10 mM linoleic acid. Specific activity of LOX was defined as U g^{-1} sample, one unit (U) was defined as 1 μmol min^{-1} of linoleic acid hydroperoxide formed at 25 °C (pH 9.0). Temperature was monitored with a Testo 174 data logger (Testo, Lenzkirch, Germany).

The activity of PPO was determined by the increment in absorbance at 398 nm for 10 s produced by the reaction between 0.5 mL of enzyme extract with 2.5 mL of 100 mM sodium phosphate buffer (pH 6.4) and 500 mM of catechol. Specific activity of PPO was defined as U g^{-1} sample, one unit (U) was defined as an increment of 0.001 in the absorbance (λ = 280 nm) per min at 25 °C (pH 6.5) in a 3.0 mL reaction mix containing L-tyrosine.

To evaluate the activity of SOD, a mixture (total volume of 3.0 mL) containing 50 mM sodium phosphate buffer (pH 7.8), 2 μM riboflavin, 75 μM NBT, 10 μM EDTA, 13 mM methionine and 0.1 mL of the extract was exposed to fluorescent light for 10 min and then the absorbance was measured at 550 nm. Identical solutions kept in the dark served as blanks. Specific activity of SOD was defined as U g^{-1} sample; one unit (U) inhibits the reduction of cytochrome *c* by 50% in a coupled system with xanthine oxidase (pH 7.8) at 25 °C.

The activity of CAT was measured by the decline in absorbance (λ = 240 nm) caused by the decomposition of H_2O_2; the reaction mixture consisted of 2.0 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.5 mL of 40 mM H_2O_2 and 0.5 mL of the enzyme extract in a total volume of 3.0 mL. Specific activity of CAT was defined as U g^{-1} sample, one unit (U) was defined as the decomposition of 1.0 μmol of H_2O_2 per min (pH 7.0) at 25 °C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM, measured by the rate of decrease of A_{240}.

The activity of POD was evaluated as follows: a mixture containing 2.0 mL of 100 mM sodium phosphate buffer (pH 6.4), 8 mM guaiacol and 0.5 mL of the enzyme extract was incubated for 5 min at 30 °C, then 1.0 mL of 24 mM H_2O_2 was added to the mixture and after 120 s the increment in absorbance at 460 nm was measured. Activity of POD was defined as U g^{-1} sample, one unit (U) of pyrogallol forming 1.0 mg purpurogallin in 20 s (pH 6.0) at 20 °C.

2.8. Determination of malondialdehyde

MDA content (μg of MDA per g of sample) was determined by measuring the absorbance at 532 nm and subtracting the non-specific absorbance at 600 nm. The mixture contained 1 mL of enzyme extract, 2 mL of 100 mM sodium phosphate buffer solution (pH 6.4) with 0.5% of TBA and 15% of TCA. This mixture was heated to 95 °C for 20 min and immediately cooled in iced water; then it was centrifuged at 12,000 rpm for 10 min and the supernatant was used for the absorbance measurements.

2.9. Sensory analysis

An untrained panel consisting of 11 assessors was used to evaluate changes in the sensory properties of the peaches during the storage. Assessors (6 females and 5 males, age 22–35) were recruited from among the staff of University of Zaragoza. The judges were familiarized with "Calanda" peach fruit, and were the same for all tests. The procedure used was the classification of the responses given by the panelists in a scale where 7 descriptors, defined according to their relevance in the final quality of the peaches, were qualified. The perception of the tasters concerning the intensity of the descriptors related to external aspect, firmness, flavor, sweetness, juiciness and off-flavor was evaluated according to a descriptive analysis in a scale ranging from 1 to 10 points, being 10 the highest intensity. "Flavor" was defined as the combined perceptions of taste and retronasal aromas; "off-flavor" is referred to the presence of strange flavors, other than those of the peach. "Acceptability" was also included as representative parameter of the global sensory quality of the peaches.

Peach fruit were peeled, sliced in small pieces and presented in balanced order for each assessment to avoid positional bias and contrast effect (Lanmond, 1977). No pre-treatment (e.g. peeling or slicing) was performed to evaluate the firmness and the external aspect of the peach fruit. For the sensory tests only those peaches without visible mold were used, and in the absence of any of these, at the last days of testing, the undamaged parts of them were selected. Panelists were asked to smell and taste the sample fruit and to evaluate (in the 0–10 scale) the external aspect, firmness, flavor, sweetness, juiciness, off-flavor and acceptability of the peaches for each treatment. The following analysis sessions were scheduled: 0, 7, 12, 16 and 20 days of storage.

2.10. Statistical analysis

The SPSS (SPSS 13.0 for Windows) statistical software was used to calculate the analysis of variance (ANOVA), Tukey's and Games–Howell tests were used. LSD means comparison test was used in figures. Significance differences were determined at the $P < 0.05$ level.

3. Results and discussion

3.1. Antifungal assays and shelf-life

Symptoms of chilling injury, with severe internal browning and mealiness of the pulp, were observed for the peaches stored at 0–0.5 °C of temperature after 30 days of storage. The effect was only visible when cutting and opening the fruit, whereas the external appearance was excellent (see supplementary figure). This behavior has been also described by other authors (Brummell et al., 2004; Jin et al., 2009). The cold damage was higher than the benefit from the active packaging and this treatment was consequently rejected. The rest of the study therefore was only focused on the tests at room temperature.

The visible microbial attack on the spoiled peach fruit was characterized by the appearance of several species of mold, mainly *Monilinia fructicola* (brown rot), *Penicillium expansum* (blue mold) and *Rhizopus* spp. (characterized by large masses of black–gray fungus extending rapidly through the entire fruit). Identification of the molds was done by direct visual inspection as the symptoms were well established.

Fig. 2 shows the results of percentage of infected fruit for the different packaging treatments. The shelf-life of the "Calanda" variety is about 3–5 days at room temperature and this was confirmed as there were 38% of infected fruit at 4 days of storage for the control packaging. Although the mean infection incidence in the active tray packaging was always lower than that of the controls, the difference in the means was only statistically significant ($P < 0.05$) on day 8 (21% for active trays vs. 69% for the controls).

There was a significant ($P < 0.05$) reduction of the infected fruit when using the active labels (Fig. 2). At 4 days of storage all the peaches were free of mold. At 8 days of storage, 7 out of 9 trays were totally free from infected fruit; for the control, 9 out of 9 trays had infected fruit (Fig. 3). At 12 days of storage there were 12.5% of infected fruit in the active label packaging vs. 86.1 and 62.5% in

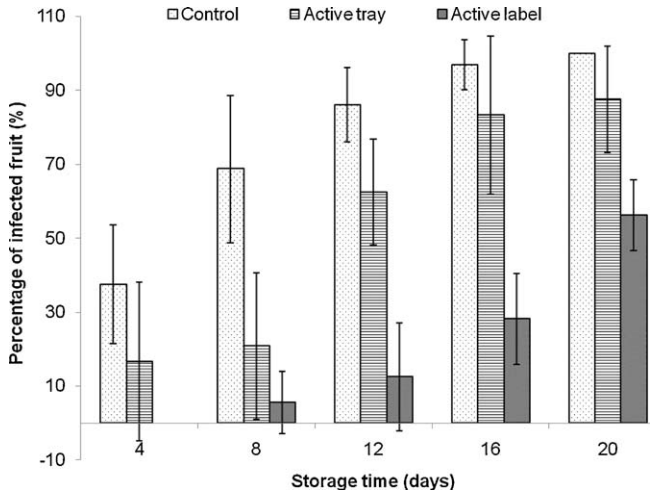

Fig. 2. Effect of the active packaging on the percentage of infected peach fruit during storage. Values represent the mean of 9 replicates/treatment.

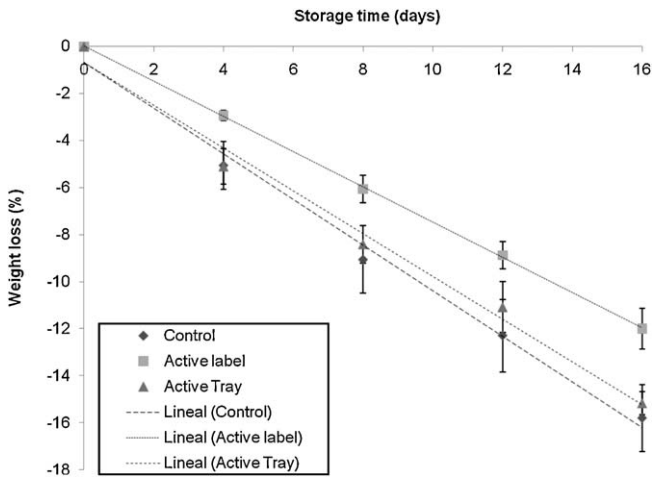

Fig. 4. Change of weight loss (percentage) during storage. Values represent the mean of 9 replicates/treatment.

the control and the active tray, respectively. Finally, at 16 days of storage, the percentage of infected fruit in the active label packaging was similar to the control at 4 days of storage and lower than the control at 8 days of storage.

3.2. Physico-chemical analysis

Weight loss is associated with increasing fruit susceptibility to fungal decay (Valverde et al., 2005). Average weight of peaches at the optimum harvested stage was 259 ± 20 g per fruit. Weight loss during the storage time was lower for the fruit stored with the active label packaging than with the active tray and the control (Fig. 4). At 16 days of storage, weight loss of peaches stored in the active label packaging was found to be 12.0% vs. 15.8% and 15.2% of the control and the active tray, respectively. No significant differences ($P < 0.05$) were observed between the control and the active tray. It has been reported that the use of vapors of main components (e.g. menthol, eugenol, thymol) of several essential oils reduced weight loss in grapes and sweet cherries (Serrano et al., 2005; Valverde et al., 2005), the mechanism of the protective effect still being unknown.

Color of the peaches was only measured until day 8 of storage because the fungal infection prevented the proper measurement in the equatorial zone of the fruit. Table 2 shows the results obtained L^*, C^* and h°. No significant differences were observed between samples stored in active and control packaging. Table 2 also shows the results of color difference (ΔE), which were established by reference to the color of the fruit at the time of harvest (day 0). As can be seen, there was an increment in the color difference during storage as a consequence of the ripening of the fruit; at 8 days of storage, the color difference in the control was 11.6 while in the active label and active tray they were 5.9 and 6.2, respectively.

Firmness of peach fruit freshly harvested was 47 ± 5 N. During storage, firmness decreased to 35 ± 3 N in the peaches stored in the control packaging, whereas it remained unchanged (51 ± 2 N) in the peaches stored in the active label packaging. The fruit firmness was

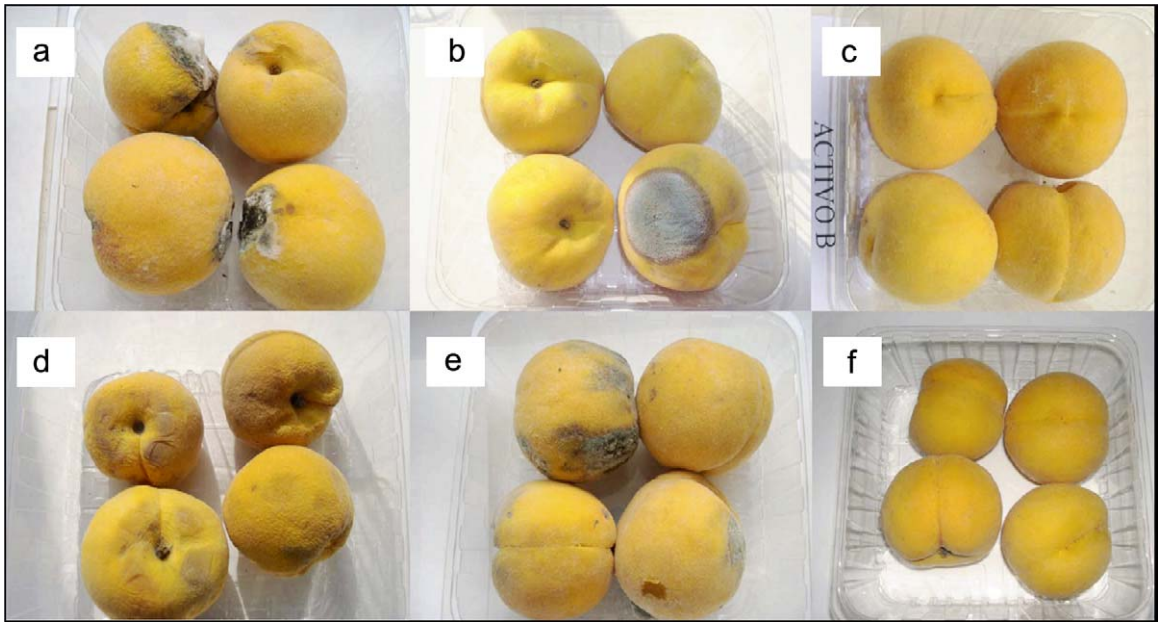

Fig. 3. One replicate of the peaches (year 2007) at 8 days of storage: (a) control, (b) active tray packaging and (c) active label packaging. Peaches at 12 days of storage: (d) control, (e) active tray packaging and (f) active label packaging.

Table 2
Effect of active packaging (label and tray) on chromaticity values (lightness, chroma, and hue angle) and color difference (ΔE) in peach fruits during the storage.

Packaging	Chromaticity values										
	Lightness (L)			Chrome (C)			Hue angle (h°)			Color difference (ΔE)	
	0 days	4 days	8 days	0 days	4 days	8 days	0 days	4 days	8 days	4 days	8 days
Control	68.8 ± 1.6	67.8 ± 2.4	65.8 ± 3.3	59.1 ± 8.5	58.3 ± 4.8	59.5 ± 5.5	181.5 ± 0.1	181.4 ± 0.5	181.4 ± 0.1	6.6 ± 3.2	11.5 ± 2.6
Active label		68.8 ± 2.2	68.8 ± 3.7		60.9 ± 6.5	58.5 ± 7.1		181.5 ± 0.1	181.4 ± 0.5	5.6 ± 3.1	5.9 ± 2.7
Active tray		71.1 ± 3.9	67.2 ± 1.9		61.2 ± 7.9	62.2 ± 6.0		181.4 ± 0.6	181.3 ± 0.6	4.3 ± 2.1	6.2 ± 2.0

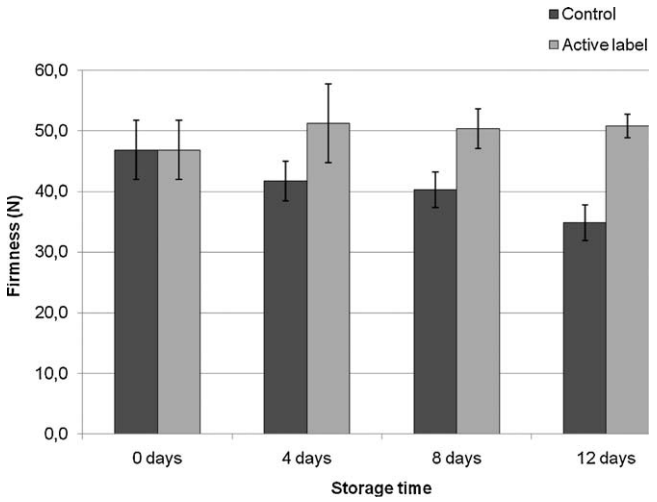

Fig. 5. Firmness (*N*) of the peach fruit in control and active label packaging during storage. Values represent the mean of 3 replicates/treatment.

significantly different ($P < 0.05$) at 12 days of storage, as can be seen in Fig. 5.

No significant differences during storage were observed for the total soluble solids content (SSC), except for the active label at 16 days of storage with an increment in SSC (Table 3). Initial values of SSC at harvest time (14.4 ± 0.7 °Brix) were higher than those published by Fernández et al. (12.5 ± 0.4 °Brix); the difference could be attributed to the time of harvest, one month later in our case (Fernández et al., 2009).

3.3. Lipoxygenase analysis

Cinnamon EO inhibited enzymatic lipid peroxidation catalyzed by LOX in the *in vitro* analysis (Fig. 6). The absorbance values of the blanks, mainly due to cinnamon EO, were 0.00, 0.291 and 0.472 for the 0, 50 and 100 μL of cinnamon EO, respectively; these values were accordingly subtracted from the absorbance curves (data not shown). As can be seen, inhibitory activity was found to be concentration-dependent. According to Naidu (1995), eugenol (the second major component of cinnamon EO) is an inhibitor of LOX-dependent lipid peroxidation, which acts as scavenger of hydroxyl radicals, blocking the formation of fatty acid hydroperoxides and thus inhibiting the propagation of lipid peroxidation. (E)-Cinnamaldehyde, the main component of cinnamon EO, also acts as a scavenger against hydroxyl radicals, with a similar percentage of radical scavenging activity to that of eugenol (Pezo et al., 2006, 2008; Singh et al., 2007). Both compounds, eugenol and (E)-cinnamaldehyde, could be responsible for the LOX catalyzed peroxidation inhibition exhibited by cinnamon EO.

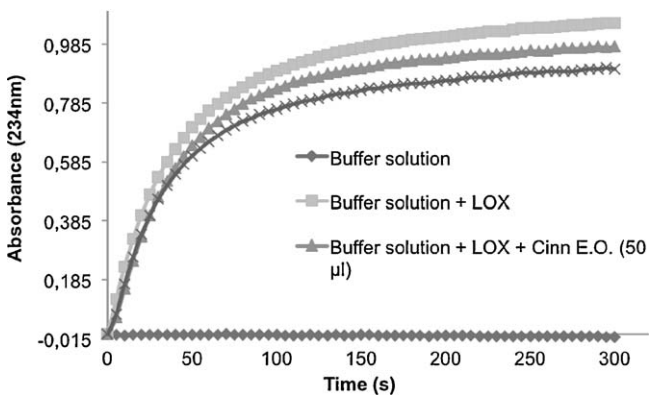

Fig. 6. Comparison of the effects of cinnamon EO (0, 50 and 100 μL) on the curve for *in vitro* LOX-catalyzed lipid peroxidation.

In vivo LOX activity in peaches increased to a maximum, and then decreased with time for both the control and the active packaging (Fig. 7A). LOX activity was statistically lower for the peaches stored with the active label packaging at 4, 8 and 12 days of storage. It is well known that LOX activity causes the peroxidation of the lipids contained in the cellular membrane, thus affecting its integrity and resulting in mechanical and chemical damage (Izzo et al., 1995). The damage of the cellular membrane together with a decrease in LOX activity could be responsible for the differences observed between active label packaging and control in weight loss and firmness. Particularly, Maalekuu et al. (2006) found a correlation between weight loss, LOX activity, membrane ion leakage and membrane lipid content in ripe pepper fruit. The decrease in LOX activity might also be concomitant with the inhibition of ethylene biosynthesis (Zhu et al., 2006).

3.4. Enzyme analysis

PPO activity decreased at the beginning of the assay and then increased to a maximum after 8, 12 and 16 days of storage for the control, the active label and the active tray, respectively (Fig. 7B). Apparently, active packaging delays maximum PPO activity; however it is not clear why this maximum started earlier with the active label. MDA content remained constant (1.1 $\mu g\, g^{-1}$ peel) during storage for the active label packaging. For the control and the active tray, MDA content dramatically increased at days 8 and 12 then dropped again (Fig. 7C). Both PPO activity and MDA content are closely related with LOX activity and are responsible for browning of peach tissues, thus the attenuation of LOX activity could be also beneficial in this sense.

CAT activity decreased over the storage time, the decrease being significantly less pronounced in the active label packaging, but also in the active tray with respect to the control (Fig. 7D). At 8 and 12 days the CAT activity was still higher (10–15 $U\, g^{-1}$ peel) in the active packaging than in the control. SOD and POD activity increased during the time to reach a plateau at 4 and 8 days of storage, respectively (Fig. 7E and F). No differences were observed except for SOD activity at 8 days of storage in the control and the active tray treatments, which was significantly lower than with the active label. SOD and POD results are not in agreement with the results from Wang et al. (2004) as they observed a decrease of the SOD and POD activity with time. As CAT, SOD

Table 3
Effect of active label packaging on soluble solids content ((°Brix) in peach fruit during the storage.

Packaging	Soluble solids content (°Brix)				
	0 days	4 days	8 days	12 days	16 days
Control	14.4 ± 0.7	14.6 ± 0.4	13.8 ± 0.5	13.9 ± 1.2	13.1 ± 0.5
Active label	14.4 ± 0.7	14.7 ± 0.5	14.4 ± 0.8	15.9 ± 0.4	18.3 ± 0.8

and POD are free-radical scavenging enzymes, their decrease may lead to high levels of reactive oxygen intermediates, thus causing lipid peroxidation and oxidative damage (Hariyadi and Parkin, 1991; Mittler, 2002). However it should be taken into account that Wang et al. (2004) inoculated the peaches with microorganisms and studied the stress response of enzymes. The results obtained here confirm the behavior of cinnamon essential oil as an antioxidant.

3.5. Sensory analysis

Fig. 8 illustrates in detail the results obtained for each packaging (control, active trays and active labels). All the descriptors were considered as positive factors except for off-flavor, which was considered as negative. As Fig. 8 shows, all the positive descriptors gave an average value above 7 at day 0 of storage, which is considered the optimum level of peach quality, thus 7 was considered as the acceptable level of quality. Off-flavor is a negative descriptor of the sensory quality of the peaches and an average value of 1.8 was obtained at day 0 of storage.

At 12 days, peaches stored in the control packaging scored very low values ranging from 3.5 to 5.5 for the positive parameters. Peaches stored 12 days in the active label packaging scored values from 6.4 to 7.4 for the positive descriptors thus in the average score considered as optimum. And most importantly, no significant differences ($P < 0.05$) were found between the peaches stored in the active packaging and the peaches at the optimum quality level, except for firmness and sweetness (Fig. 8). From these results it may be concluded that the active label packaging improved the quality sensory characteristics of the peaches compared to the non-

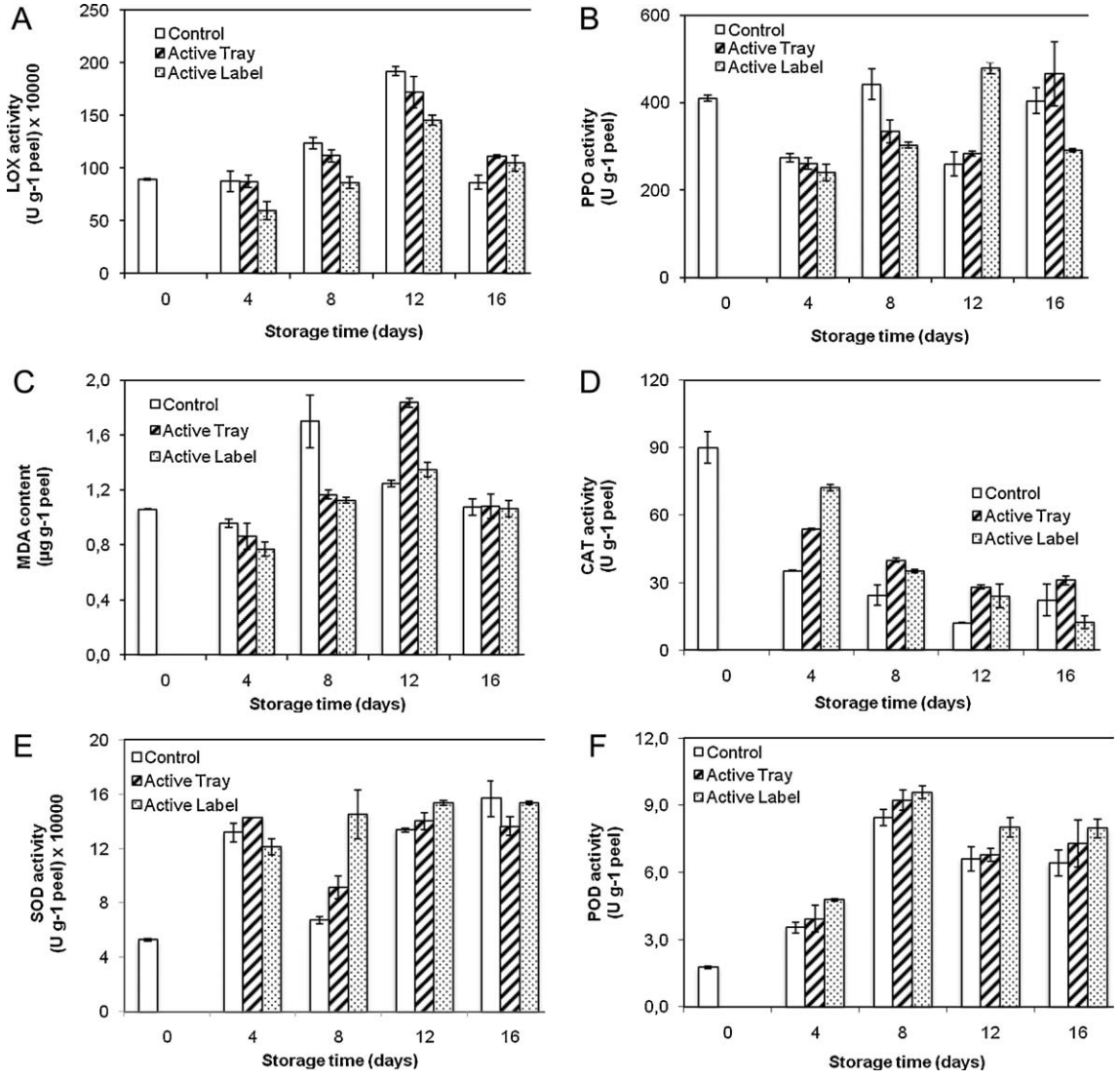

Fig. 7. LOX, PPO, CAT, SOD and POD activities and MDA content in peach fruit during storage. Values represent the mean of 3 replicates/treatment.

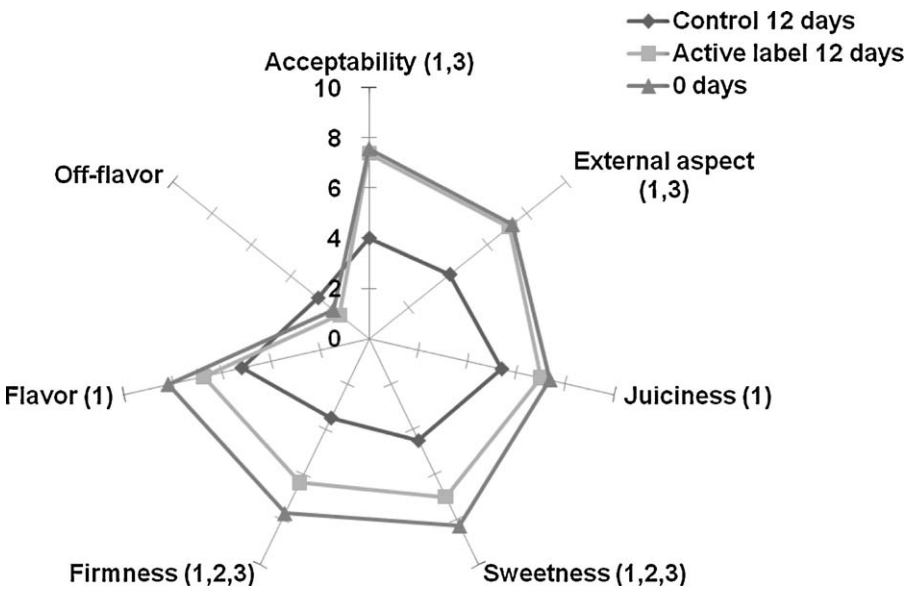

Fig. 8. Sensory scores of peach fruit stored in control and active packaging at 0 and 12 days of storage. Significant differences ($P < 0.05$) are labeled with numbers: 1 – difference between 0 days and control 12 days; 2 – difference between 0 days and active label 12 days; and 3 – control 12 days and active label 12 days.

active packaging. Moreover, the acceptability, which is considered the representative parameter of the global sensory quality of the peaches, scored a value (7.4) very close to that scored by the peaches at the optimum quality (7.5).

Negative parameters such *as off-flavor* scored very low both in the active (1.5) and the non-active packaging (2.6). None of the panelists reported cinnamon flavor in any of the samples. Peach fruit are less susceptible to changes in their sensory properties as they are peeled before being eaten.

3.6. Conclusions

Several conclusions can be drawn from the experimental work reported here. First, the use of a self-adhesive active label placed inside a macro-perforated tray of PET offers an efficient option for extending the shelf-life of peach fruit at room temperature. This type of active packaging is more efficient than the use of an active macro-perforated tray of PET, probably due to the higher concentration of active compounds released by the packaging. Second, some physico-chemical properties such as weight loss and firmness were also better when active label-based packaging was used. It has been demonstrated as well that cinnamon EO, the active agent, inhibits the activity of lipoxygenase, and the use of the active packaging somehow influences the activity of some selected enzymes. All these enzymes participate in the oxidation processes and the changes in their activity are in all cases related to antioxidant protection. This fact, supported by the experimental data, confirms once again the efficient antioxidant properties of cinnamon essential oil. Finally, as a result of all these factors, global sensory quality of the fruit peach at 12 days of storage at room temperature was similar to that at the optimum quality level. No cinnamon or off-flavor was detected by the panelists in any sample, which is an important finding.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.postharvbio.2011.01.008.

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