DOI:10.1021/jf100728n

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Active Paraffin-Based Paper Packaging for Extending the Shelf Life of Cherry Tomatoes

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A new active paraffin coating for paper and board was evaluated for antimicrobial protection and decay retardation for cherry tomatoes. Different active agents were evaluated against *Alternaria alternata* fungus both in vitro and in vivo using artificially inoculated cherry tomatoes. Bark cinnamon and oregano essential oil showed the best performance (versus clove and leaf cinnamon essential oils) when incorporated to active paper or board used for packaging at nominal concentrations of 3 and 6% (w/w), respectively. Almost total inhibition of the fungus was obtained when 6% of bark cinnamon essential oil was applied to the packaging material. A number of physicochemical parameters such as pH, weight loss, water activity, and color were monitored, and no significant differences between active, blank, and control samples were found for weight loss and color difference. The maximum transfer of *trans*-cinnamaldehyde and carvacrol to the food was detected after 1 or 2 days of storage. Sensorial analysis was performed, and panelists were not able to detect changes in cinnamon-based packaged tomatoes but they could in the oregano-based tomatoes.

KEYWORDS: Active paper packaging; cherry tomato; essential oils; *Alternaria alternata*

INTRODUCTION

The use of essential oils to control spoilage of tomatoes has been reported by some authors. Holt et al. used garlic extracts and extract fractions to check their antimycotic activity in vitro and in planta (*1*). Tzortzakis used eucalyptus and cinnamon essential oils to control the postharvest quality of strawberry and tomato fruit, and the results suggested that the essential oil vapor may improve fruit quality attributes in addition to the antimicrobial protection (*2*). Thyme, sage, nutmeg, eucalyptus, and cassia essential oils were also used by Feng et al. to control *Alternaria alternata* both in vitro and in vivo (*3*). More recently, cassia oil has been used in combination with magnesium sulfate to control the postharvest storage roots of cherry tomatoes (*4*). All of these reports have been made by direct use of the essential oils, posing a clear risk for organoleptic alteration of the food.

A new active packaging based on the incorporation of natural essential oils to paraffin used as a coating in paper and board has recently been proposed (PCT patent WO2007144444-A1). Two different paraffin formulations can be used as vehicle to incorporate the essential oils: pure solid paraffin waxes and paraffin emulsions intended to be applied to paper followed by a water evaporation step. Cinnamon essential oil showed the highest antimicrobial activity, and the active paraffin was shown to be more efficient in the fungal than in the bacterial inhibition (*5*). The use of cinnamon essential oil against microbial spoilage (*Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *Aspergillus niger*) of fresh produce has been recently reported (*6*).

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A first design of this active paper packaging tested with real food has been reported, and the effectiveness of the proposed alternative in the inhibition of *R. stolonifer* in sliced bread after 10 days of storage was demonstrated (*7*). *trans*-Cinnamaldehyde has been identified as the key chemical for antimicrobial activity responsible for the antimicrobial properties, as has been previously reported (*8*−*10*). However, the incorporation rate of the *trans*-cinnamaldehyde to the foodstuff is unclear, although it is suspected than inhibition takes place in the early hours after storage. Also, the influence of the active compounds in the organoleptical properties of the bread was not evaluated, this being a major concern when essential oils are used as active agents.

The main aim of this work is to develop the active packaging to assess if it is still effective when used in high water content foodstuffs, such as fruits and vegetables. Cherry tomato (*Lycopersicon esculentum*) was selected as vegetal to evaluate the active paper packaging. It is a seasonal crop, and its availability is limited during the whole year (*3*); however, it can be easily stored because it has a small size, thus allowing several experiments to be performed at laboratory scale. Cherry tomato is susceptible to suffer from postharvest disease caused by several fungal pathogens, the major ones being *A. alternata*, *B. cinerea*, and *R. stolonifer* (*11*, *12*). As the target microorganism, an *A. alternata* pathogenic fungus was selected. Estimates of tomato crop losses can range as high as 90% for some diseases associated with *Alternaria* spp. (*1*, *13*). Additionally, many strains of *Alternaria* are known to produce mycotoxins (*14*, *15*).

The work described herein was conducted in several steps. The first one was to design an appropriate alternative of active packaging to inhibit the growth of *A. alternata*, and in vitro antimicrobial

 Published on Web 05/17/2010

tests were performed for this purpose. In a second step, the best alternatives were tested with cherry tomatoes artificially inoculated with *A. alternata*. Decay evaluation and some physicochemical parameters of the tomatoes were controlled. In a third step, the concentrations of *trans*-cinnamaldehyde and carvacrol, which are the major active agents in bark cinnamon and oregano essential oils, respectively, were determined in the fruit during the storage time. The final step of the procedure was to perform a sensorial analysis of the cherry tomatoes stored in the active packaging.

MATERIALS AND METHODS

Plant Material and Essential Oils. Cherry tomatoes ('Lupita' variety, size = 23−28 mm) at the commercially ripe stage were obtained from a local market and selected according to apparent/visual uniformity. Only tomatoes free from injuries were selected and, before testing, tomatoes were randomized to avoid bias. Essential oils from the following plant species were used as active agents in this work: *Cinnamomum zeylanicum* (cinnamon), *Origanum vulgaris* (oregano), and *Syzygium aromaticum* (clove). Two different cinnamon essential oils were tested: cinnamon leaf oil, which has eugenol (> 70%) as the main component, and bark cinnamon oil, which consists of *trans*-cinnamaldehyde (75−85%) among others (*16*). All of the essential oils were supplied by Argolide Química (Barcelona, Spain).

Chemicals. Carvacrol (5-isopropyl-2-methylphenol, Chemical Abstracts Service (CAS) Registry No. 499-75-2) was obtained from Fluka (Madrid, Spain). *trans*-Cinnamaldehyde (CAS Registry NO. 14371-10-9) was purchased from Aldrich (Madrid, Spain). Ethanol (HPLC grade) was obtained from Scharlab (Barcelona, Spain), and distilled water was obtained by using a Milli-Q model plus 185 water purified from Millipore (Bedford, MA). A stock solution (approximately 1900 μg/g of each analyte) was prepared in ethanol. Working solutions used to fortify tomato samples in the standard addition procedure were prepared by appropriate dilution of the stock solution with ethanol.

Microbial Culture. *A. alternata* (Colección Española de Cultivos Tipo CECT 2662, Valencia, Spain) strain was stored at −18 °C in sterilized skimmed milk. Fungal conidia were harvested after inoculation on Sabouraud agar medium for 7 days at 25 °C and transferred to a test tube with physiological saline solution; further dilutions (500 μL into 4.5 mL) to final working concentrations were also done in physiological saline solution. No separation of conidia and mycelia fragments was conducted.

Active Paper and Packaging Manufacture. The active paper manufacture was as follows: active paraffin formulations (based on both solid and emulsion paraffin) containing the appropriate amount of the essential oil or the pure *trans*-cinnamaldehyde were prepared by Rylesa-Repsol (Madrid, Spain) in a joint project with the University Research Group GUIA. This methodology is protected by PCT patent WO2007144444-A1. The concentration of the active agent was varied as described in the text.

For the solid paraffin, the manufacturing process involves a mixing step of heating at about 110 °C for 5 min in a closed vial. Active coatings were then manually applied to Kraft paper [100 g/m^2] provided by Rylesa-Repsol using a heated manual coating bar supplied by RK Print-Coat Instruments Ltd. (Litlington, U.K.).

For the paraffin emulsions the manufacturing process does not include a heating step, and coating of the paper is done with an automated coating machine K Control Coater supplied by RK Print-Coat Instruments Ltd. Coating was applied on both sides of the Kraft paper, although only one side had the active formulation. After the coating, coated paper was left to dry (10 min) at room temperature. Grammage was controlled by weighting according to the wax manufacturer's specifications (7−9 g/m^2 for emulsions and 18 g/m^2 for solid paraffin). The sheets of active paper were prepared 24 h prior to the tests and stored in bags of polypropylene, in contact with each other and simulating an industrial winding.

Active papers were used to make packaging containers for storage. These containers were made emulating the plastic commercial ones, with similar dimensions of 10.5 × 13 × 6 cm (820 cm^3 of volume and 555 cm^2 of surface). The size of the packaging box allows the storage of up to 18 cherry tomato units (250 g). Thus, the cherry tomato mass/active paper surface ratio was 0.45 g/cm^2.

Antifungal Assays. In vitro antifungal assays in Petri dishes were carried out as follows: a Petri dish with the appropriate solidified agar culture medium (Sabouraud chloramphenicol) was inoculated with a sterilized platinum transfer loop dipped in an *A. alternata* conidial suspension (see Microbial Culture). Then, the active coated paper was placed over the Petri dish and nonhermetically kept in place using a plastic strap. Controls (with paper coated by the paraffin formulation but without the active ingredients) were also prepared for each set of samples. Plates were incubated for 7 days at 25 °C. Fungal growth was monitored after 7 days by measuring the radial growth. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. All of the experiments were performed four times.

For in vivo testing, cherry tomatoes were wounded with a sterile puncher to make a uniform wound of about 3 mm wide on their peel at the top region. Then, a sterilized platinum transfer loop dipped in a conidial suspension of *A. alternata* was used to inoculate each wounded tomato. Inoculated tomatoes were stored at ambient temperature (about 21 °C) in the paper packaging boxes. Five replicates for active packaging and seven for the controls and the blanks (commercial plastic packaging) were analyzed. The visible microbial attack on the spoiled tomato was characterized as a dark spot first, then evolving to visible mold and, eventually, in the rip of the tomato itself. The severity of deterioration was visually evaluated after 5 and 10 days of storage. The degree of infection on fruit was rated using a scale of 1−5, where 1 = no infection, 2 = trace infection, 3 = small infection, 4 = moderate infection, and 5 = severe infection (*2*, *17*) (see the Supporting Information). The value assigned to each package is calculated from the individual values of the tomatoes and using the equation

$$v_t = \Sigma n_i v_i / n_t \quad (1)$$

where v_t is the value assigned to the whole packaging, n_i is the number of tomatoes with i value (from the scale), v_i is the value assigned to the individual fruits in the 1−5 scale, and n_t is the total number of tomatoes in the packaging.

Physicochemical Analysis. To evaluate the effect of the active packaging on the cherry tomatoes, some parameters (weight loss, pH, color, and water activity) were monitored before treatment and during storage. Five fruits from each experiment were weighted at specific time intervals (0, 3, 5, 7, and 10 days of storage). The results were expressed as percentage of weight loss compared to the initial weight.

The pH of the cherry tomatoes was determined by using a PH25 pH-meter (Crison, Barcelona, Spain) equipped with a puncture electrode, suitable for measurements in foodstuffs. Three tomatoes were measured in each replica, and each treatment was repeated five times.

The color of the cherry tomatoes was measured with a CR-400 chroma meter (Konika Minolta, Tokyo, Japan). Color changes were quantified in the CIELAB color space. L^* refers to the lightness, chroma [$C^* = (a^{*2} + b^{*2})^{0.5}$] represents color saturation, and hue angle ($h°$) = $\tan^{-1}(b^*/a^*)$ when $a^* < 0$ and $b^* > 0$ or $h° = 180° + \tan^{-1}(b^*/a^*)$ when $a^* > 0$ and $b^* > 0$, is defined as a color wheel (*4*, *18*). Ten fruits were measured in each of the five replicas. Although C^*, $h°$, and L^* magnitudes are used to measure the absolute color, it is much more interesting to determine the color differences. For this reason, the CIE 1976 color difference [$\Delta E = (\Delta L^{*2} + [\Delta a^{*2} + [\Delta b^{*2})^{0.5}$] 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; ΔL^*, Δa^*, and Δb^* are the lightness difference and a^* and b^* differences for the two independent measures (*19*).

Water activity of cherry tomatoes was evaluated with a LabMASTER-aw (Novasina, Lachen, Switzerland) water activity instrument equipped with a temperature-stabilized measurement chamber. Tomato samples were blended before measuring. Chamber settings were as follows: temperature, 25 °C; stabilization observation time for water activity, 3 min; stabilization observation time for temperature, 1 min.

Quantification of *trans*-Cinnamaldehyde and Carvacrol in Tomato. A procedure for the determination of *trans*-cinnamaldehyde and carvacrol (among others) from tomato samples has been used (*20*). Tomato samples were chopped with a knife and homogenized for 2 min at 2400 rpm in an Ultra-Turrax T18 blender obtained from IKA (Staufen, Germany). An aliquot of each sample (3 g) was weighed into the SPME glass vial, made up with 1.5 g of Milli-Q water, and sealed with the PTFE cap. The vial was stabilized at 55 °C, and a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was exposed to

the headspace generated by the stirred (500 rpm) sample inside the vial for 40 min at 55 °C. After extraction, the loaded fiber was automatically transferred to the injection port of the GC-MS chromatograph, where desorption takes place for 5 min at 250 °C. A standard addition method is used to avoid the matrix effect. The whole procedure has proven useful, providing detection limits in the low micrograms per kilogram range and wide and convenient linear ranges (*20*).

GC-MS determinations were performed using a Hewlett-Packard model 6890N GC (Agilent Technologies, Santa Clara, CA) equipped with a 5975B inert XL mass spectrometric detector and a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), which allows automatic SPME analysis; data were collected and processed using the MSD ChemStation software (Agilent Technologies).

A HP-5MS (Agilent Technologies) analytical column of 30 m × 0.25 mm and 0.25 μm film thickness was used. The temperature program for the gas chromatography was as follows: initial temperature, 45 °C, held for 1 min, linear gradient of 2 °C/min to 85 °C, second ramp at 5 °C/min to 170 °C, and final ramp at 15 °C/min to 200 °C. The injector temperature was 250 °C, and injection was performed in the splitless mode (splitless time, 30 s). The carrier gas was helium (99.999% purity, 1.0 mL/min) supplied by Carburos Metálicos (Barcelona, Spain). Compounds of interest, *trans*-cinnamaldehyde and carvacrol, were then quantified by SIM mode, once their characteristic masses had been identified from their full spectra.

Sensorial Analysis. An untrained panel consisting of 12 assessors evaluated the cherry tomatoes during the storage (1, 3, 5, and 10 days). A triangular difference test was performed to find out if the active packaging somehow modifies the flavor of the tomatoes; each assessor evaluated one set of three samples, two identical and one different. Panelists were asked to smell and taste the samples, to identify the odd sample, and to describe the difference of flavor on a sheet. Samples were presented in balanced order for each assessment to avoid positional bias and contrast effect (*21*). Analysis of the results of the triangle test were based on significance tests (*22*).

Statistical Analysis. SPSS (SPSS 13.0 for Windows) statistical software was used to calculate the analysis of variance (ANOVA), and Tukey's and the Games−Howell test were used in tables. LSD mean comparison test was used in figures. Significance differences were determined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

In Vitro Antifungal Assay. The antimicrobial activity of the manufactured active paper against *A. alternata* with nominal concentration ranging from 2 to 8% (w/w) of the active agent incorporated into the coating was evaluated ($n = 4$). *A. alternata* was fully inhibited when using 3% (w/w) of *trans*-cinnamaldehyde as the active agent in the emulsion (**Figure 1a**). The use of bark cinnamon essential oil also inhibited the *A. alternata* growth, but nominal concentration as high as 7% was necessary to obtain an inhibition of 96%. No significant inhibition was reported when cinnamon leaf oil and oregano and clove essential oils were used as active agents in the emulsion, even at the maximum concentration tested of 7%. Differences in the activity of the two cinnamon oils tested, bark and leaf, can be attributed to the amount of *trans*-cinnamaldehyde in its composition (see Plant Materials and Essential Oils).

Better results were obtained using active solid paraffin as coating in the paper. *A. alternata* was fully inhibited with all of the essential oils tested at the highest concentration of the active agent (**Figure 1b**). Stronger inhibition was obtained again with bark cinnamon oil and *trans*-cinnamaldehyde as active agents; 3% (w/w) nominal concentration in the paraffin completely inhibited *A. alternata*. Complete inhibition was also obtained by using oregano, cinnamon, and clove at 5, 8, and 8% (w/w) nominal concentrations, respectively.

A number of conclusions can be inferred from this experiment: First is that the active paper manufactured with the essential oils tested has activity against *A. alternata*. Second, solid paraffin coating is a better way to incorporate active agents to the packaging than the paraffin emulsion. Third, bark cinnamon oil, pure

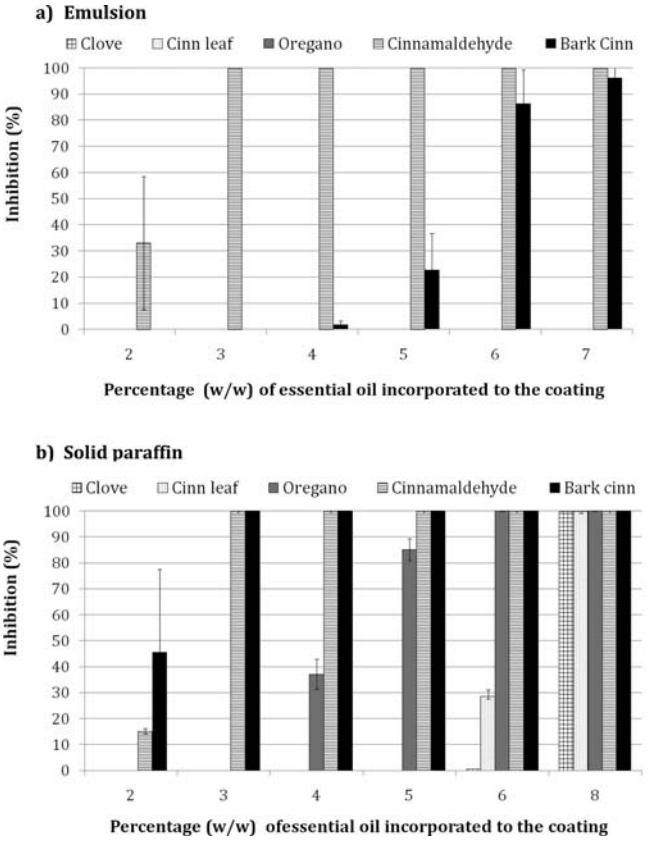

Figure 1. *Alternaria alternata* growth inhibition as a percentage of radial growth relative to the control with (**a**) emulsion based active coating and (**b**) solid paraffin based active coating. Values represent the mean of four replicates per paper packaging.

trans-cinnamaldehyde, and oregano essential oil have the strongest antimicrobial activities against *A. alternata*. Quite interesting is that bark cinnamon essential oil gave slightly better results than *trans*-cinnamaldehyde when solid paraffin was used, whereas *trans*-cinnamaldehyde provided higher activity when using aqueous emulsion.

The explanation of these results could be in the manufacturing process: active paper with solid paraffin includes a heating step at 110 °C for 5 min leading to degradation and losses of *trans*-cinnamaldehyde; on the other hand, the protective effect of eugenol and other compounds present in the cinnamon essential oil against thermal degradation of *trans*-cinnamaldehyde has been reported by Friedman et al. (*23*), which could be why the antimicrobial activity for pure *trans*-cinnamaldehyde is stronger in aqueous solution paraffin (with no heating step, but with an evaporation step). Moreover, volatile active agents in the active paraffin emulsion could be partially lost during the drying step after paper coating. Finally, Lopez et al. have demonstrated the synergic effect in the inhibition activity of some minor components of the essential oils, such as eugenol (*24*). This synergic effect does not happen when pure *trans*-cinnamaldehyde is used as active agent.

In Vivo Antifungal Assay. On the basis of the experiments above-discussed, solid paraffin was used hereafter as coating for the active paper; bark cinnamon essential oil (3 and 6% w/w nominal concentration), pure *trans*-cinnamaldehyde (3 and 6%), and oregano essential oil (5.5%) were selected as active agents to perform in vivo antifungal assays on cherry tomato.

Results of deterioration showed that significant protection against infection was obtained when using oregano, bark cinnamon, and *trans*-cinnamaldehyde at 6% (w/w) of nominal concentration

Table 1. Effects of Active Paper Packaging Based in Bark Cinnamon Essential Oil (Cinn EO), Pure *trans*-Cinnamaldehyde, and Oregano EO (Oreg EO) (% w/w Nominal Concentration) on Visible Decay, pH, Water Activity, Color Difference (ΔE), and Color (Lightness, Chroma, and Hue Angle) in Cherry Tomato Fruits during Storage[a]

	visible decay			pH			water activity	ΔE
cherry tomato	0 days	5 days	10 days	0 days	5 days	10 days	10 days	10 days
blank	1.0 a	1.6 abc	2.0 c	4.2 a	4.3 ab	4.6 cd	0.91 ± 0.0	
control		1.4 abc	1.8 bc		4.4 abc	4.6 d	0.88 ± 0.02	1.9 ± 1.5
cinn EO (3%)		1.3 ab	1.5 abc		4.3 ab	4.5 bcd	0.87 ± 0.01	2.8 ± 1.7
cinn EO (6%)		1.0 a	1.0 a		4.4 abc	4.4 abcd	0.85 ± 0.02	3.4 ± 1.8
cinnamaldehyde (3%)		1.3 ab	1.5 abc		4.4 abcd	4.4 abcd	0.87 ± 0.01	3.8 ± 1.4
cinnamaldehyde (6%)		1.1 a	1.1 a		4.3 ab	4.4 abc	0.85 ± 0.01	4.7 ± 1.5
oreg EO (5.5%)		1.2 ab	1.5 abc		4.3 ab	4.4 abc	0.89 ± 0.04	3.8 ± 1.5

	lightness			chroma			hue angle ($h°$)		
cherry tomato	0 days	5 days	10 days	0 days	5 days	10 days	0 days	5 days	10 days
blank	36 a	36 a	36 a	28 a	27 a	27 a	52 a	50 a	50 a
control		36 a	36 a		27 a	27 a		52 a	49 a
cinn EO (3%)		36 a	36 a		26 a	26 a		51 a	51 a
cinn EO (6%)		37 a	36 a		28 a	27 a		50 a	54 a
cinnamaldehyde (3%)		37 a	37 a		27 a	29 a		51 a	50 a
cinnamaldehyde (6%)		36 a	37 a		29 a	28 a		48 a	55 a
oreg EO (5.5%)		37 a	37 a		26 a	28 a		51 a	50 a

[a] For each parameter, values followed by the same letter do not differ significantly (p <0.05) according to the significance tests.

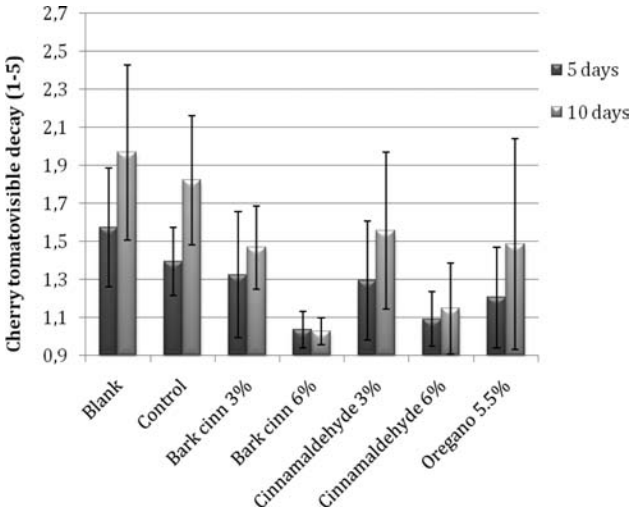

Figure 2. Cherry tomato visible decay (on a 1−5 scale) of the different active (and nonactive) packaging at 5 and 10 days of storage. Values represent the mean of five to seven replicates/treatment.

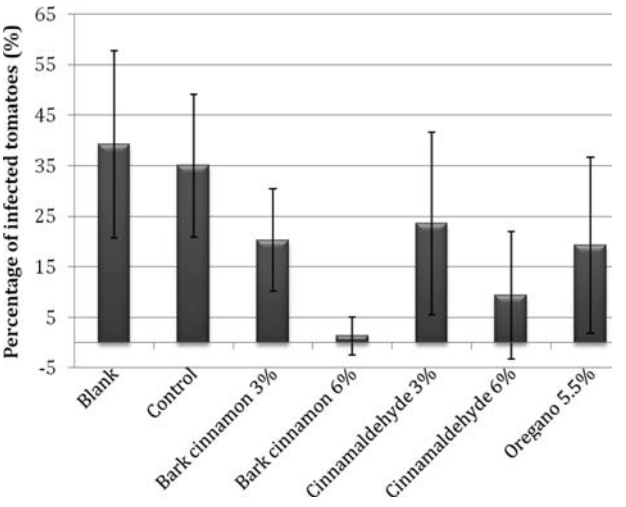

Figure 3. Effect of the active packaging in the percentage of infected tomatoes after 10 days of storage. Values represent the mean of five to seven replicates/treatment.

at 10 days of storage (**Table 1**; **Figure 2**). No significant differences between blanks, control, and 3% (w/w) concentration of essential oils in the coating were observed. At 5 days of storage no significant differences were observed between active and nonactive packaging.

The results can also be expressed as percentage of infected fruit. The percentage of infected fruits was recorded after 10 days of storage. As can be seen in **Figure 3**, the percentage of infected tomatoes was significantly reduced by bark cinnamon essential oil 6% in the paraffin. Blank and control showed very similar results and for the rest of packaging, reduction of infected fruits was also observed, but the reduction was not statistically different because of the poor reproducibility of the decay.

Physicochemical Analysis. Weight loss at 10 days was found to be higher, 4.7−6.9%, than obtained in previous studies, which was around 1.3% (*2*). This difference could be attributed to the tomato wounds allowing higher respiration rates than unwounded tomatoes and also because of the differences in storage temperature. No significant differences ($p < 0.05$) were found between the active and nonactive packaging (**Figure 4**) despite blank and control weight losses being slightly higher than with active packages. This tendency has been observed as well in previous works using cinnamon essential oil as active agent. Moreover, weight loss values were more reproducible for nonactive packaging with RSD of < 10% versus 19.8−25.5%. Some authors have reported that the use of vapors of eugenol, thymol, or menthol can reduce weight loss in cherries and grapes (*25*), but this effect has not been observed in this case.

The increment of pH of the tomatoes stored in active packaging was slightly lower than that of tomatoes stored in nonactive packaging, blank, and control, as **Table 1** shows. Statistical differences ($p < 0.05$) were found at 10 days of storage for the tomatoes in the blank and control packaging compared with the fresh tomatoes at 0 days. pH values are comparable to those reported in the literature (*2*). It is thought that pH has no marked influence on the growth of fungal species; however, this factor can greatly influence the formation of mycotoxins (*26*).

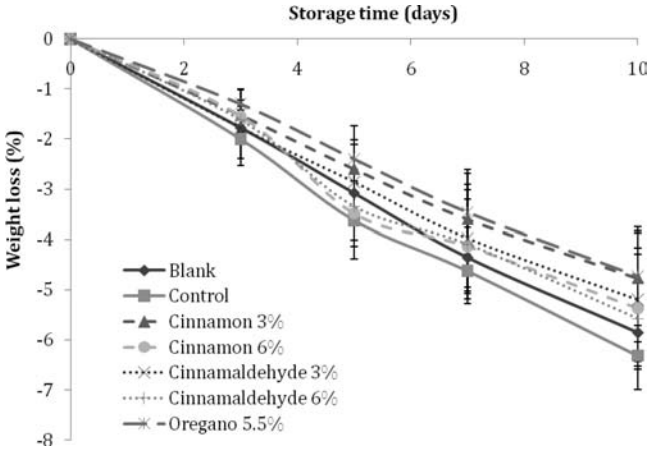

Figure 4. Evolution of weight loss (percentage) over storage time. Values represent the mean of five to seven replicates/treatment.

Water activity of tomato diminished during the storage (data not shown), and after 10 days of storage a_w of tomatoes packaged with cinnamon and *trans*-cinnamaldehyde essential oil based active paraffin was lower ($p < 0.05$) than the blanks (**Table 1**). No significant differences were observed between active and controls. For the plastic commercial packaging, a_w values were higher than for wax paper packaging. Moreover, a_w values both in 6% cinnamon essential oil and in pure *trans*-cinnamaldehyde were very close to 0.85−0.88, which is the minimal water activity for germination and growth of *A. alternata* (*26*, *27*). Surprisingly, a_w values for fresh tomato were quite different from those reported in the literature (0.985) (*28*). Thus, these data should be treated with caution.

Color changes during storage were not observed. **Table 1** shows the results obtained for L^*, C^*, and $h°$. No significant differences were observed between samples stored in active and nonactive packaging, nor for samples along the duration of the experiment. **Table 1** also shows the results of color difference. After 5 days of storage, the differences between the tomatoes packed in the active paper and packaged in the commercial container are in the range of 1.7−3.5 CIELAB units (data not shown). At 10 days, the differences rise in all cases except in the control and are in the range of 1.9−4.7 CIELAB units. All active packages except that with 3% cinnamon have a $\Delta E > 3$. Melgosa et al. established that differences above 5.0 CIELAB units indicate significant differences in color (*29*). From an industry perspective, between 2.8 and 5.6 we can speak of a normal tolerance. In our case, none of the differences exceeds 5.6 units.

Quantification of *trans*-Cinnamaldehyde and Carvacrol in Tomato. **Figure 5** shows the results obtained over the storage time. Similar trends were observed during the time: there is a maximum at a few hours of storage, and then the active compound amount starts to drop. As can be seen, the amount of carvacrol in cherry tomato is much higher than that of *trans*-cinnamaldehyde (about 100-fold). These results are quite surprising, especially taking into account that the antimicrobial capacity of the oregano active packaging is less than that of the cinnamon one (see In Vitro Antifungal Assay and In Vivo Antifungal Assay). Differences could be attributed to the different affinities of carvacrol and *trans*-cinnamaldehyde into the mixture of waxes of the cuticular membrane of the cherry tomato fruits (*30*), as can be seen in terms of the octanol/water partition coefficients, 1.82 and 3.49 for *trans*-cinnamaldehyde and carvacrol, respectively.

For the *trans*-cinnamaldehyde amount, there is a maximum concentration at 2 days of storage, with 432 μg/kg for the 6% bark cinnamon essential oil active paper and 245 μg/kg for the 3% bark cinnamon essential oil. Cherry tomatoes stored with oregano

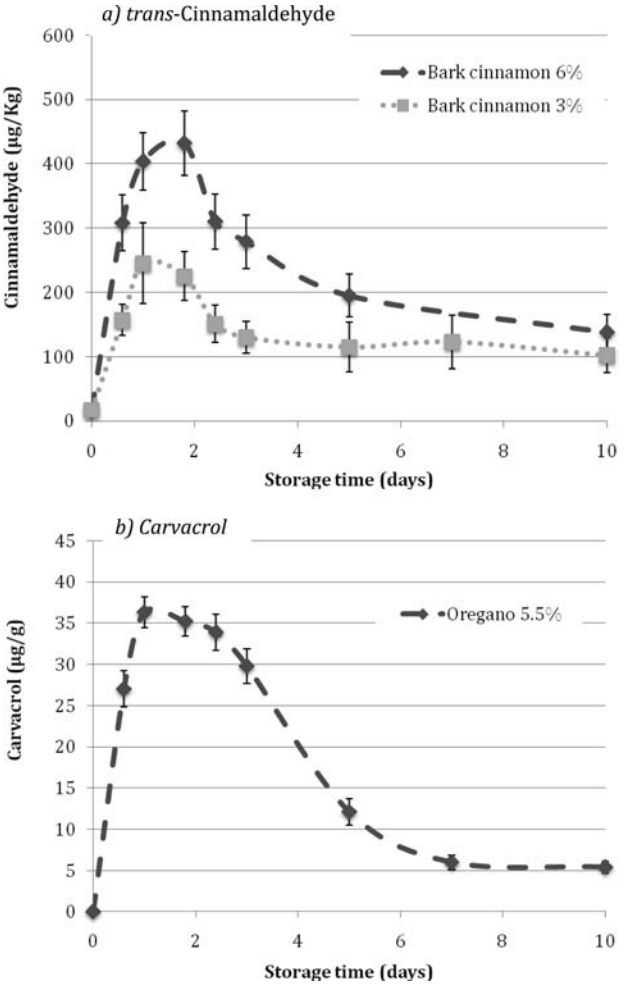

Figure 5. *trans*-Cinnamaldehyde (**a**) and carvacrol (**b**) amounts found in cherry tomatoes in μg/kg and μg/g, respectively, during storage with bark cinnamon 3 and 6% (w/w) and oregano 5.5% (w/w).

essential oil active paper (5%) had a maximum of carvacrol concentration of 36.3 μg/g.

The amount of *trans*-cinnamaldehyde in cherry tomatoes after 3 days of storage is also very low (279 μg/kg, bark cinnamon 6% (w/w)) compared to the 60 μg/g obtained in bread stored for 3 days in bark cinnamon 6% (w/w) active paper reported in a previous paper (*7*). The difference in the packaging could be based on this great difference of *trans*-cinnamaldehyde amount in the food. Slices of bread were wrapped with paper in a sealed package similar to commercial packaging, so that the generation and maintenance of an active atmosphere are fostered. In addition, the loaf is wrapped in the paper, so the distance to the active container is minimal. Cherry tomatoes were packed in waxed paper boxes that are not tight closed, with side vents so the cherry tomatoes can breathe, and it is more difficult to maintain an active atmosphere. Kraft paper is also very porous and promotes the loss of volatiles.

Moreover, although the percentages of cinnamon essential oil incorporated in both packages are the same, the paper used, the paraffin formulation, and the thickness of the coating are different. Another factor that may explain the difference in concentrations lies in the type of food being packaged. The bread, due to its fluffiness, has a high specific surface and has many points of contact with the atmosphere through which the active compounds can penetrate. The tomato has a lower surface/food ratio, it is a more closed system, and, therefore, the amount that actually

Table 2. Sensorial Tests Results Based on a Total of 12 Judgments (12 Panelists × 1 Judgment)[a]

		storage time			
		1 day	3 days	5 days	10 days
bark cinnamon essential oil 6%	accurate answers	6	5	1	2
	cinnamon flavor	4	4	0	0
	significant differences ($p < 0.05$)	no	no	no	no
oregano essential oil 5.5%	accurate answers	9	9	5	3
	oregano flavor	11	9	5	2
	significant differences ($p < 0.05$)	yes	yes	no	no

[a] The level at which it is considered that there are differences in the test of significance (p <0.05) is eight accurate answers.

enters the tomato is small compared to the total feed, which is what is considered in the analysis. Finally, the affinities of *trans*-cinnamaldehyde for the tomatoes and bread matrices should be different, thus being major factors in the concentration of the active agent in the foodstuff.

Sensorial Evaluation of the Tomatoes. No significant differences ($p < 0.05$) were reported when fortified cinnamon-based active paper was used as packaging. However, significant differences were obtained when oregano was used as active agent at 1 and 3 days of storage. **Table 2** shows the number of accurate answers over the storage time. For the active paper with cinnamon essential oil as active agent, 4 of 6 accurate answers were associated with cinnamon flavor at 1 day of storage and 4 of 5 at 3 days of storage. At 5 and 10 days of storage none of the samples was related with cinnamon flavor and accurate answers were by probability. For the oregano-based active paper, all of the panelists described a strong flavor of oregano and bad taste (even those who did not provide accurate answers in the test) at 1 day of storage.

As can be seen, these results are in strong agreement with the carvacrol and *trans*-cinnamaldehyde amounts found in cherry tomato and described under Quantification of *trans*-Cinnamaldehyde and Carvacrol in Tomato. The profile obtained is identical to the profiles obtained for the amount of active compounds. In both cases, the maximum is reached at 1−3 days of storage, and then there is a drop of active compound concentration and accurate answers, respectively. This correspondence verifies that the highest amount of essential oil compounds (*trans*-cinnamaldehyde, carvacrol, ...) can somehow modify the flavor of the storage food when relatively high concentrations are used.

In conclusion, from the study carried out we can emphasize that the active paraffin-based paper packaging is a very useful approach to extend the shelf life of cherry tomato. The concentration of the active agents such as bark cinnamon and oregano essential oils should be under control. Cinnamon EO is most recommended because it requires less concentration in the packaging than oregano EO to get the required results, thus minimizing the risk of sensorial change.

ABBREVIATIONS USED

PCT, Patent Cooperation Treaty; CAS, Chemical Abstracts Service; HPLC, high-performance liquid chromatography; SPME, solid-phase microextraction; PTFE, polytetrafluoroethylene; DVB, divinylbenzene; CAR, carboxen; PDMS, polydimethylsiloxane; GC, gas chromatography; MS, mass spectrometry; SIM, selected ion monitoring; ANOVA, analysis of the variance; RSD, relative standard deviation; EO, essential oil.

ACKNOWLEDGMENT

We acknowledge the active paraffin supplied by Rylesa-Repsol.

Supporting Information Available: Pictures of the cherry tomatoes stored in the active packaging and in the commercial packaging, of the cherry tomato decay scale (1−5), of the cherry tomatoes with the control, blank, and active packaging at 10 days of storage and a table with the concentrations of the main components of the essential oils (EOs) used as active agents. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Holt, D. L.; Gomez-Almonte, N. Anti-mycotic activity of garlic extracts and extract fractions in vitro and in planta. *J. Food Prot.* **1995**, *58*, 322–325.

(2) Tzortzakis, N. G. Maintaining postharvest quality of fresh produce with volatile compounds. *Innovative Food Sci. Emerg. Technol.* **2007**, *8*, 111–116.

(3) Feng, W.; Zheng, X. Essential oils to control *Alternaria alternata in vitro* and *in vivo*. *Food Control* **2007**, *18*, 1126–1130.

(4) Feng, W.; Zheng, X.; Chen, J.; Yang, Y. Combination of cassia oil with magnesium sulphate for control of postharvest storage rots of cherry tomatoes. *Crop Prot.* **2008**, *27*, 112–117.

(5) Rodriguez, A.; Batlle, R.; Nerin, C. The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. *Prog. Org. Coat.* **2007**, *60*, 33–38.

(6) Tzortzakis, N. G. Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. *Innovative Food Sci. Emerg. Technol.* **2009**, *10*, 97–102.

(7) Rodriguez, A.; Nerin, C.; Batlle, R. New cinnamon-based active paper packaging against *Rhizopus stolonifer* food spoilage. *J. Agric. Food Chem.* **2008**, *56*, 6364–6369.

(8) Kim, H. Y.; Young, J. L.; Ki, H. H.; Yong, K. K.; Kyu, C. S.; Ju, Y. L.; Hong, Y. C.; In, S. K.; Sang, B. H.; Chul, W. L.; Il, S. S.; Jae, S. C. Isolation of antimicrobial substances from natural products and their preservative effect. *Food Sci. Biotechnol.* **2001**, *10*, 59–71.

(9) Ouattara, B.; Simard, R. E.; Piette, G.; Bégin, A.; Holley, R. A. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int. J. Food Microbiol.* **2000**, *62*, 139–148.

(10) Lopez, P.; Sanchez, C.; Batlle, R.; Nerin, C. Development of flexible antimicrobial films using essential oils as active agents. *J. Agric. Food Chem.* **2007**, *55*, 8814–8824.

(11) Lee, J. P.; Lee, S. W.; Kim, C. S.; Son, J. H.; Song, J. H.; Lee, K. Y.; Kim, H. J.; Jung, S. J.; Moon, B. J. Evaluation of formulations of *Bacillus licheniformis* for the biological control of tomato gray mold caused by *Botrytis cinerea*. *Biol. Control* **2006**, *37*, 329–337.

(12) Schena, L.; Ippolito, A.; Zahavi, T.; Cohen, L.; Nigro, F.; Droby, S. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. *Postharvest Biol. Technol.* **1999**, *17*, 189–199.

(13) Akhtar, K. P.; Matin, M.; Mirza, J. H.; Shakir, A. S.; Rafique, M. Some estudies on the post harvest diseases of tomato fruits and their chemical control. *Pakistan J. Phytopathol.* **1994**, *6*, 125–129.

(14) Harwing, J.; Scot, P.; Stoltz, D.; Blanchfield, B. Toxins of molds from decaying tomato fruit. *Appl. Environ. Microbiol.* **1979**, *38*, 267–274.

(15) Stinson, E. E.; Osman, S. F.; Heisler, E. G.; Siciliano, J.; Bills, D. Mycotoxin production in whole tomatoes, apples, oranges, and lemons. *J. Agric. Food Chem.* **1981**, *29*, 790–792.
(16) Thomas, J.; Duethi, P. Cinnamon. In *Handbook of Herbs and Spices*; Woodhead Publishing: Cambridge, U.K., 2001; p 326.
(17) Wang, C. Y.; Buta, J. G. Maintaining quality of fresh-cut kiwifruit with volatile compounds. *Postharvest Biol. Technol.* **2003**, *28*, 181–186.
(18) McGuire, R. G. Reporting of objective color measurements. *HortScience* **1992**, *27*, 1254–1255.
(19) Meléndez-Martínez, A. J.; Vicario, I. M.; Heredia, F. J. Correlation between visual and instrumental colour measurements of orange juice dilutions: effect of the background. *Food Qual. Pref.* **2005**, *16*, 471–478.
(20) Rodriguez-Lafuente, A.; Nerín, C.; Batlle, R. Determination of fifteen active compounds released from paraffin-based active packaging in tomato samples via microextraction techniques. *Anal. Bioanal. Chem.* **2009**, *395*, 203–211.
(21) Lanmond, E. *Laboratory Methods for Sensory Evaluation of Food*; Food Research Institute: Ottawa, Canada, 1977.
(22) Roessler, E. B.; Warren, J.; Guymon, J. F. Significance in triangular taste tests. *Food Res. Int.* **1948**, *13*, 503–505.
(23) Friedman, M.; Kozukue, N.; Harden, L. A. Cinnamaldehyde content in foods determined by gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 5702–5709.
(24) Lopez, P.; Sanchez, C.; Batlle, R.; Nerin, C. Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against foodborne microorganisms. *J. Agric. Food Chem.* **2007**, *55*, 4348–4356.
(25) Valverde, J. M.; Guillen, F.; Martinez-Romero, D.; Castillo, S.; Serrano, M.; Valero, D. Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. *J. Agric. Food Chem.* **2005**, *53*, 7458–7464.
(26) Samson, R. A.; Hoekstra, E. S.; Frisvad, J. C. *Introduction to Food- and Airborne Fungi*, 7th ed.; CBS − Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2004; p 389.
(27) Frisvad, J. C.; Samson, R. A. Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production. In *Handbook of Applied Mycology*; Dekker: New York, 1991; pp 31−68.
(28) Heredia, A.; Barrera, C.; Andrés, A. Drying of cherry tomato by a combination of different dehydration techniques. Comparison of kinetics and other related properties. *J. Food Eng.* **2007**, *80*, 111–118.
(29) Melgosa, M.; Pérez, M. M.; Yebra, A.; Huertas, R.; Hita, E. Algunas reflexiones y recientes recomendaciones internacionales sobre evaluación de diferencias de color. *Opt. Appl.* **2001**, *34*, 1–10.
(30) Leide, J.; Hildebrandt, U.; Reussing, K.; Vogg, G. The developmental pattern of tomato fruit wax accumulation and its impact on cuticular transpiration barrier properties: effects of a deficiency in a β-ketoacyl-coenzyme A synthase (LeCER6). *Plant Physiol.* **2007**, *144*, 1667–1679.

Received for review February 22, 2010. Revised manuscript received May 7, 2010. Accepted May 10, 2010. This work has been financed by Project PETRI PET-2006-0438 from the Spanish Ministry of Education and Science. A.R.-L. gratefully acknowledges the Aragón Regional Government and the European Social Fund for a grant (ref B055/2005).