2240 *J. Agric. Food Chem.* **2007,** *55,* 2240–2245

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Active Package for Wild Strawberry Fruit (*Fragaria vesca* L.)

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An antimicrobial active package has been developed to improve the safety and quality of wild strawberries, as well as extending their shelf life. The fruits were packed in equilibrium-modified atmosphere packaging (EMAP), and the effect on *Botrytis cinerea* growth and on the quality parameters of the fruit by the addition of different amounts of 2-nonanone, an antifungal volatile compound naturally present in strawberries, was investigated during storage at 10 and 22 °C. The temperature of 10 °C was chosen as the temperature used at points of sale, and 22 °C was chosen as the control temperature. Fungal growth was inhibited in all cases, possibly due to the synergistic effect of high CO_2 partial pressures and the presence of the antifungal compound. Weight, soluble solids, titrable acidity, and anthocyanin losses were retarded by the presence of 2-nonanone. This effect was more pronounced as the 2-nonanone concentration was increased at both temperatures. Therefore, an active package that releases 2-nonanone inhibits fungal decay and delays the senescence of highly perishable wild strawberry fruit.

KEYWORDS: Antifungal active packaging; wild strawberry fruit; 2-nonanone; EMAP

INTRODUCTION

The natural appearance, color, and nutritional value of wild strawberries (*Fragaria vesca* L.) are characteristics that make these fruits highly appreciated by consumers. However, the postharvest preservation of wild strawberries is very complex due to a very fast metabolism and the presence of microbial contamination, which cannot be reduced by external washing, as in other fresh products, due to an extremely sensitive surface. Thus, at room temperature, the shelf life of wild berries is less than 48 h.

Different strategies to reduce postharvest losses such as combinations of low temperature, high-humidity storage, and the use of carbon dioxide have proven to be effective (*1*). Several reports have associated their effect on fruits (including cultivated berries) with reductions in dehydration, respiration, and ethylene production rates (*2*).

To extend the marketing period, it is necessary to limit fungal infection, mainly caused by *Botrytis cinerea*. This can be achieved by several treatments such as applying coatings to the surface or storing the fruit in a controlled or modified atmosphere to minimize the deteriorative losses (*3*−*6*). Modified atmosphere packaging has proven to be among the most efficient of these treatments. An appropriate package reduces product transpiration and respiration, protects against mechanical damage, improves the appearance, and prolongs shelf life for a few days. When the package is not well-designed, however, the CO_2 content increases to levels that are efficient at inhibiting fungal growth but trigger the accumulation of off-flavors responsible for sensorial deterioration (*7*, *8*).

Previous studies (*2*, *9*) have shown that an atmosphere of about 10% CO_2 and 10% O_2 is suitable for prolonging the storage of wild strawberries and that this atmospheric composition can be obtained at 10 °C by modified atmosphere packaging (MAP) with microperforated films. Nevertheless, fruit decay was observed after long-term storage, especially at room temperature.

Active packaging is a novel food technology in which the packaging system plays an active role in the maintenance of food quality and safety. Generally, the package contains an active component that interacts beneficially with the food or the internal gaseous environment, resulting in an extension of shelf life. Active packaging technologies include antimicrobial packaging systems, in which the package actively inhibits mold and/or bacteria growth, generally by releasing an additive with antimicrobial properties. This agent can be either immobilized within the package structure or incorporated into a substrate, pad, or sachet.

Several substances have been recognized and used as microorganism growth inhibitors. Among these, special attention is being paid to substances that are naturally present in fresh produce. This is the case of several organic compounds, which are constituents of strawberry aroma (*10*−*12*). Specifically, 2-nonanone is an antifungal compound with low mammalian toxicity [oral rate $LD_{50} = 3200$ mg/kg (*13*)], a pleasant, fruity/floral odor, resistance to rapid decomposition, adequate volatility, environmental acceptability, and a high potential for commercial development, besides retarding and preventing the decay of whole fruit (*14*).

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10.1021/jf062809m CCC: $37.00

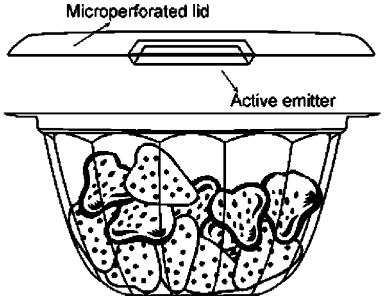

Figure 1. Active packaging system for wild strawberry fruit.

The present paper focuses on the development of an active package that releases 2-nonanone as a natural antifungal compound to prolong the shelf life of wild strawberries. The effect of 2-nonanone concentration was monitored through the evolution of selected fruit freshness parameters.

MATERIALS AND METHODS

Materials. Wild strawberries (*F. vesca* L., Reina de los Valles) at the red-ripe stage, grown in Canals (Valencia, Spain), were harvested in the early morning and transported to the laboratory within 1 h in a refrigerated vehicle. Damaged, nonuniform, unripe, or overripe fruits were eliminated, and the selected fruits were stored for at least 2 h at 3 °C to ensure equilibrium.

Packaging Material. Polypropylene (PP) (125 cm^3)/ethylene-vinyl alcohol copolymer/PP cups (300 μm wall thickness) supplied by Huhtamaki (Nules, Castellon, Spain) were used for packaging approximately 40 g of wild strawberries (20 fruits). Fifty micrometer thick heat-sealable polyethylene terephthalate/PP lids with three microperforations were used to thermoseal these cups (Amcor Flexible, Bristol, United Kingdom). Pores presented an average diameter of 100 μm. A previous study demonstrated that this package provided an adequate equilibrium atmosphere for this product (*8*).

Metallocene polyethylene (mPE) was selected as the film material to manufacture the active bags due to the high permeability of this material to organic compounds. The antimicrobial compound was included in an adsorbent solid. Previous tests revealed the high sorption capacity of alumina. mPE sachets containing 0.1 g of alumina F-1 80/100 mesh (Supelco, Bellefonte, PA) (per sachet) impregnated with 0, 2.5, 5, and 10 μL of 2-nonanone (Sigma-Aldrich Chemie, Steinheim, Germany) were prepared shortly before use. Powder and liquid were placed in a vial, which was closed and mixed with vortex. They were used as the active emitter of the packaging system and were fixed to the inner surface of the lid prior to cup sealing as shown in **Figure 1**.

Methods. *Sample Preparation.* Cups were filled with 20 wild strawberries, sealed with lids with 2-nonanone emitters, and stored at 10 °C and 77% relative humidity (RH) or 22 °C and 50% RH. At 0, 1, 2, and 3 days of storage, 6 cups per 2-nonanone concentration were evaluated as follows: Three were used to measure color and headspace composition (in triplicate), and the other three were mixed and blended to obtain a purée. This purée was then used to measure the remaining quality parameters. Each package was weighed before opening. Sixty wild strawberries were chosen at random to evaluate quality characteristics on day 0.

Weight Loss. The net and gross weights of each package were measured on day 0 with a Voyager analytical balance (Ohaus, Suiza). The gross weight was also recorded during storage. Values were monitored as percentage of weight loss per initial fruit weight.

Headspace Composition. The headspace composition in each package was analyzed by gas chromatography. Before opening, an adhesive septum (Lippke-Handels, Neuwied, Germany) was stuck on the lid surface and a 100 μL sample was withdrawn from the package headspace and injected into the injection port of a Hewlett-Packard 5890 series II gas chromatograph (GC) (Agilent Technology, Barcelona, Spain) equipped with a thermal conductivity detector (TCD) and a Chromosorb 102 column (Restek, Teknokroma, Barcelona, Spain). Helium was the carrier gas. The injector, oven, and detector temperatures were 100, 32, and 100 °C, respectively. The GC was previously calibrated by analyzing known amounts of pure and mixed gases supplied by Abello-Linde (Valencia, Spain).

Titrable Acidity. Six grams of the strawberry purée was diluted with 100 mL of distilled water and filtered to remove the pulp. The acidity, expressed as mg citric acid/100 mL juice, was measured by titration with 0.1 N NaOH to an end point of pH 8.1. The pH value was determined using a pH 526 WTW pHmeter (Merck, Barcelona, Spain) with a glass electrode. Three measurements were done per sample.

Soluble Solids Content. The total soluble solids content of the strawberry purée was measured with an Atago RX-1000 digital refractometer (Atago Co. Ltd., Tokyo, Japan). Three measurements were performed by sample. The results were expressed as °Brix.

Color. To measure total color, anthocyanins were extracted by the method of Sanz et al. (*15*), and the content was estimated by measuring the absorbance at 517 nm of the ethanolic extract, using a Hewlett-Packard Diode array 8452A spectrometer (Agilent Technology). Results were expressed as nmol pelargonidin 3-glucoside per gram of strawberry.

Off-Flavors and 2-Nonanone Content. The contents of three fermentative metabolites (acetaldehyde, ethyl acetate, and ethanol) and 2-nonanone during storage with different concentrations of the active compound were monitored by GC−flame ionization detection (FID) using a procedure optimized in a previous work (*2*).

Samples of 2.5 g of strawberry purée were placed in 10 mL vials, crimp-sealed, and frozen at −20 °C. For GC analysis, samples were thawed out at room temperature for 20 min and heated at 50 °C for 20 min. The volatile compounds were extracted immediately by solid-phase microextraction (SPME) using a 65 μm PDMS/DVB SPME fiber (Supelco Inc., Barcelona, Spain). The fiber was exposed to the vial headspace for 20 min, and the trapped volatiles were immediately desorbed (for 5 min) at the splitless injection port of a GC Hewlett-Packard 5890 series II (Agilent Technology) equipped with FID and a Rtx-1301 column (0.50 μm × 0.53 mm, 30 m, Restek, Teknokroma). The oven temperature was initially 40 °C for 5 min and was then heated to 200 °C at 5 °C/min and maintained for 2 min. The injector and detector temperatures were 240 °C. Three vials per treatment were analyzed. Quantification was performed after calibrating the GC-SPME system by the addition method. A set of 6 g of strawberry puree samples was supplemented with increasingly known amounts of the volatile compounds and were analyzed following the procedure previously described (*8*).

The concentration of 2-nonanone in package headspace was also determined by GC. A sample of 100 μL of the gas was sampled and injected using the chromatographic conditions described above. The GC was previously calibrated by the injection of known amounts of 2-nonanone.

Fungal Decay. The presence of *B. cinerea* was visually estimated in each individual fruit immediately after opening the packages. Wild strawberry fruits showing surface mycelial development were considered decayed. The results were expressed as percentage of fruits infected by *Botrytis*.

Statistical Analysis. The StatGraphics Plus program version 2.1 (Statistical Graphics Corp., United States, 1994−1996) was used for the analysis of variance and to test significant differences between means with $p \leq 0.05$.

RESULTS AND DISCUSSION

Weight Loss. Water loss involves a negative effect on the appearance of wild strawberry fruit, leading to shriveling and a dull-looking epidermis. The maximum permissible water loss for strawberries before marketability is impaired has been reported to be approximately 6% (*16*). Nevertheless, in a previous work on the optimization of modified atmosphere packaging for strawberries, evidence of dehydration was observed at 3% water loss (*8*). **Figure 2** shows the weight loss of strawberries packaged with different amounts of 2-nonanone for 3 days at 10 and 23 °C. As can be seen, the water loss was below these limits in all cases.

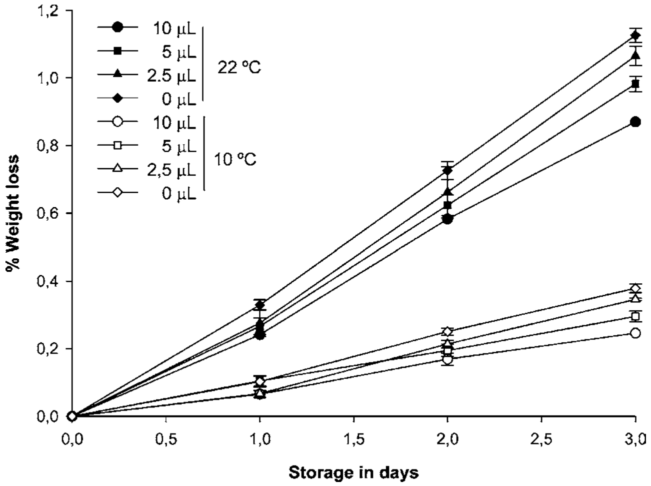

Figure 2. Weight loss of wild strawberries packaged with different amounts of 2-nonanone and stored at 10 or 22 °C.

Table 1. CO_2 and O_2 Percentages Reached in Packages of *F. vesca* with Different Amounts of 2-Nonanone after Storage for 3 Days at 10 or 22 °C[a]

	% CO_2 equilibrium		% O_2 equilibrium	
2-nonanone (μL)	10 °C	22 °C	10 °C	22 °C
0	10 aα	23 aβ	15 aα	5 aβ
2.5	8 bα	25 abβ	16 aα	4 abβ
5	8 bα	23 aβ	16 aα	5 aβ
10	9 abα	26 bβ	15 aα	3 bβ

[a] The letters a and b mean significant differences ($p < 0.05$) between CO_2 and O_2 levels reached in packages with different amounts of 2-nonanone. α and β mean significant differences ($p < 0.05$) caused by temperature for the same amount of 2-nonanone.

Table 2. Soluble Solid Content (°Brix) and Titrable Acidity (mg Citric Acid/100 mL Juice) of *F. vesca* Packaged with Different Amounts of 2-Nonanone after 3 Days of Storage at 10 or 22 °C[a]

	soluble solid content		titrable acidity	
2-nonanone (μL)	10 °C	22 °C	10 °C	22 °C
day 0	11.06 A	11.06 A	1.22 A	1.22 A
day 3				
0	10.37 aαB	9.17 aβB	0.83 aαB	0.58 aβB
2,5	10.48 bαB	9.28 bβB	0.86 bαB	0.60 bβB
5	10.84 cαB	9.52 cβB	0.90 cαB	0.65 cβB
10	10.85 cαB	9.62 dβB	0.97 dαB	0.79 dβB

[a] The letters a, b, c, and d mean significant differences ($p < 0.05$) between °Brix or titrable acidity values reached in packages with different amounts of 2-nonanone. α and β mean significant differences ($p < 0.05$) caused by temperature for the same amount of 2-nonanone. A and B mean significant differences ($p < 0.05$) in °Brix or titrable acidity values before and after treatment.

Storage temperature had an intense effect ($p < 0.05$) on the weight loss of packaged berries. At the ambient storage temperature (22 °C), after 3 days of storage, the fruit presented losses of between 0.87 and 1.13%. This contrasts with the values reached at the low storage temperature (10 °C), which were four times lower (0.24−0.38%). This temperature effect, which was expected, was caused mainly by a higher RH in the external environment resulting in less water being lost through package permeation. As a consequence of this reduction, a decrease in the transpiration rate of the fresh produce occurs since the internal RH equilibrates faster with that of the fruit.

In general, the presence of 2-nonanone occasioned a reduction of water loss, which was more pronounced as the amount of compound was increased ($p < 0.05$). Thus, the active packaging with the addition of 2.5, 5, and 10 μL of 2-nonanone resulted in a reduction in water loss, as compared to MAP strawberries, of 5, 13, and 23%, respectively, at 22 °C and day 3. At 10 °C and day 3, the reductions were 11, 24, and 37%, respectively.

CO_2/O_2 Headspace Composition. The effect of the active emitter on the composition of the equilibrium-modified atmosphere reached inside the packages was also measured at both storage temperatures; the results are presented in **Table 1**. All packages reached an equilibrium atmosphere during the first 12 h of storage.

As shown in **Table 1**, CO_2 values of 8−10% at 10 °C and 23−26% at 22 °C were obtained after 3 days of storage, indicating a significant temperature effect on the equilibrium CO_2 concentrations ($p < 0.05$). With respect to oxygen, the equilibrium concentrations were 15−16% at 10 °C and 3−5% at 23 °C. The main reason for this temperature effect in both gases is that the increase in the respiration rate of fresh produce caused by the higher temperature is not counterbalanced by a similar increase in the package permeation rate (*17*). However, the presence of 2-nonanone has little or no effect on the equilibrium-modified atmosphere of packaged berries. Therefore, in view of the CO_2 and O_2 contents, the permeation of the packaging materials to respiration gases should not be modified to correct the effect of the active agent.

Titrable Acidity. Titrable acidity was expressed as mg citric acid/100 mL juice, as this is the predominant organic acid in strawberries (*4*). As shown in **Table 2**, wild strawberries in active packages with different 2-nonanne concentrations showed a decrease in acidity during storage, although this depended on both the temperature and the amount of 2-nonanone. The reduction in titrable acidity of berries in MAP has been observed previously and is commonly related to maturation (*8*, *18*, *19*). This relation also explains the larger decrease observed at 22 °C. In fact, independently of the 2-nonanone amounts tested, the decrease in temperature (from 22 to 10 °C) reduced the fall in citric acid at the end of the storage by 30% ($p < 0.05$).

However, the most interesting feature is the effect of 2-nonanone on titrable acidity. The larger the amount of active agent in the emitter is, the lower the reduction of titrable acidity is, the effect being observed at both temperatures. Thus, after 3 days of storage, the presence of 2-nonanone reduced the decreases in acidity from 52 to 35% at 22 °C and from 32 to 20% at 10 °C. Because increasing maturity has been related to levels of citric acid decrease (*19*), the antifungal compound appears to delay the senescence of wild strawberry fruits.

Soluble Solid Content. The soluble solid content of wild strawberries decreased during storage in both passive and active packages (**Table 2**). This observed reduction, which could mostly be explained by the hydrolysis of sucrose and the utilization of the corresponding reducing sugars in fruit respiration, was in agreement with previous results for MAP of strawberries (*8*, *18*, *20*). As the results show, both the storage temperature and the amount of 2-nonanone significantly affected ($p < 0.05$) the reduction in soluble solid content during storage. As expected, fewer sugars were consumed at lower temperatures, which improved postharvest preservation. However, the presence of 2-nonanone also affected the changes in this parameter. The lower decrease in soluble solids caused by 2-nonanone could be further evidence of a delay in the maturation process and, therefore, of better preservation of wild strawberries.

Anthocyanin Content. Anthocyanins are responsible for the color of strawberry fruit. Of these compounds, pelargonidin 3-glucose (P3-G) is the major anthocyanin in strawberry fruit

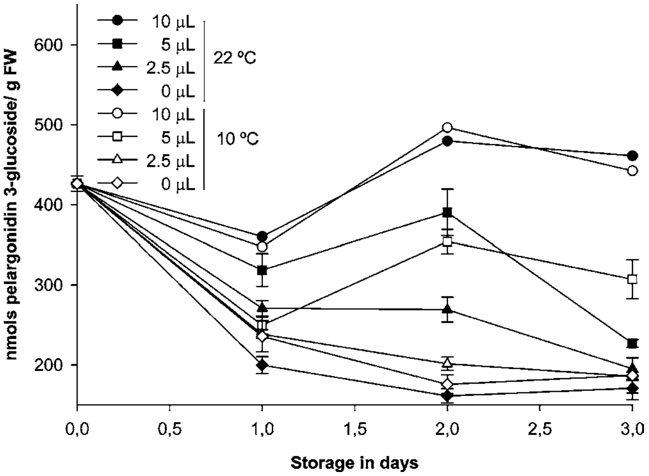

Figure 3. Effect of active packaging on anthocyanin content (P3-G/g FW) of *F. vesca* stored at 10 or 22 °C.

(≈90%) (*5*) and was consequently selected as the parameter to measure color evolution in this study.

Figure 3 presents the evolution of anthocyanin content over the storage period at 10 and 22 °C with different concentrations of the active compound. Besides a general decrease in the concentration of P3-G during storage, as described by Cordenunsi et al. (*21*) in cultivated strawberries, the clearest observation was that the storage temperature did not induce significant differences ($p < 0.05$) in anthocyanin concentration. In contrast, the concentration of 2-nonanone clearly modified the evolution of this quality parameter.

According to these results, the presence of 2-nonanone reduced the effect of storage time on fruit color. After 3 days of storage at 10 or 22 °C, the concentration of P3-G in the fruit varied significantly ($p < 0.05$) with the quantity of 2-nonanone in the active emitter. The higher the volume of the compound added is, the lower the loss of anthocyanin in the fruit is, which could be related to a delay in the fruit ripening process.

Fermentative Metabolites. The quality of wild strawberries is generally related to their aroma, which is the result of a complex multicomponent relationship among many aromatic constituents (*22*). Changes in aroma are generally caused by accumulation of fermentative metabolites such as acetaldehyde, ethanol, and ethyl acetate during storage (*23*).

In this study, 4, 37, and 3 mg/kg acetaldehyde, ethanol, and ethyl acetate concentrations were measured in wild strawberries at harvest, which proved to be quite similar to those obtained by Larsen and Watkins (*24*) for cultivated strawberries. During storage, the increase in the fermentative volatiles content was more pronounced at room temperature than in refrigeration ($p < 0.05$). This evolution in MAP has been described previously in *Fragaria ananassa* (*15*, *20*).

Table 3 also shows that the concentration depended on the amount of 2-nonanone ($p < 0.05$) placed in the emitter. As can be seen, after 3 days of storage, the strongest off-flavors were detected in wild strawberries packaged without 2-nonanone (control). In these conditions (MAP), the acetaldehyde content increased eight-fold at 10 °C and 10-fold at 22 °C in relation to the content at harvest. These increases were diminished by the insertion of the active device. Thus, active packages with 2.5, 5, and 10 μL of 2-nonanone showed acetaldehyde reductions of 12, 24, and 48% at 10 °C and 20, 28, and 45% at 22 °C, respectively. Because Smagula and Bramlage link the accumulation of acetaldehyde with physiological breakdown caused by over-ripeness (*25*), the exogenous addition of 2-nonanone could result in a delay in strawberry ripening.

Table 3. Changes in Concentrations (mg/kg) of Acetaldehyde, Ethanol, and Ethyl Acetate Reached Inside Wild Strawberry Packages with Different Amounts of 2-Nonanone after 3 Days of Storage at 10 or 22 °C[a]

	mg volatile/kg fruit					
	acetaldehyde		ethanol		ethyl acetate	
2-nonanone (μL)	10 °C	22 °C	10 °C	22 °C	10 °C	22 °C
day 0	4	4	37	37	3	3
day 3						
0	33 aα	40 aβ	78 aα	130 aβ	21 aα	64 aβ
2.5	29 bα	32 bβ	76 abα	174 bβ	22 aα	71 bβ
5	25 cα	29 cβ	74 bα	146 cβ	19 aα	59 cβ
10	17 dα	22 dβ	61 cα	135 aβ	15 bα	49 dβ

[a] Letters a, b, c, and d mean significant differences ($p < 0.05$) in mg volatile/kg FW reached in packages with different amounts of 2-nonanone. α and β mean significant differences ($p < 0.05$) caused by temperature for different amounts of 2-nonanone.

Similar effects were observed in the evolution of the ethanol and ethyl acetate contents during storage in the active package, although the reduction in the accumulation of these two compounds was only significant at the highest 2-nonanone additions. Pyruvate decarboxylase and alcohol dehydrogenase are two important enzymes responsible for acetaldehyde and ethanol production (*26*); because these volatiles were reduced by the insertion of 2-nonanone, this antifungal volatile could be associated with a delay in the enzymatic activity of both and, therefore, in wild strawberry fruit off-flavor production. However, this hypothesis relating the volatile concentrations with enzymatic activity should be further investigated.

2-Nonanone Content. 2-Nonanone is a naturally occurring compound, so the exogenous addition of small amounts of this compound should not have any toxicological effect on consumers. Nonetheless, the concentration of 2-nonanone absorbed by the wild strawberries was analyzed to provide assurances that the intake value was far below the oral toxicity value of $LD_{50} = 3200$ mg/kg (*13*).

Figure 4 presents the evolution of 2-nonanone concentration in the headspace and in the fruit over the 3 days of storage at 10 and 22 °C with and without the inclusion of the active device. The concentration of 2-nonanone in the headspace reached a maximum value during the first or second day in all samples. This profile is the result of several mass transfer processes, which take place simultaneously: the desorption from the sachet, the sorption in the fruit and in the package walls, and the permeation out of the package through the pores. As can be seen in **Figure 4**, the headspace concentration varied between 0.5 and 0.05 mg/L depending on the amount of 2-nonanone introduced in the sachet and the storage temperature. These concentrations were much lower than those tested by Vaughn et al. (*14*) to check the antimicrobial effect of this compound on fungal decay of strawberries.

Regarding the fresh produce, the initial concentration of 2-nonanone was 3 mg/kg of fresh weight. This value decreased slightly during storage, with a more intense fall at the higher temperature ($p < 0.05$). With the active packaging, the concentration of 2-nonanone increased due to sorption by the fruit. The largest increase occurred during the first day of storage and decreased during the rest of the storage time, probably due to loss of the compound by permeation through the package. This profile is also in agreement with the headspace concentration evolution. The concentration in the product depended on

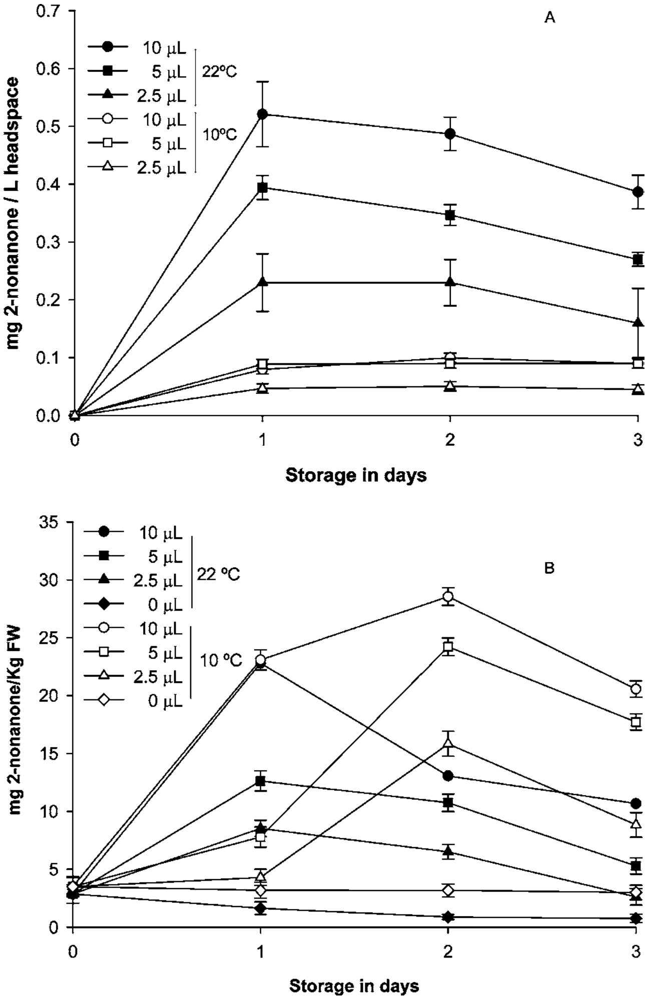

Figure 4. Evolution of 2-nonanone concentration in the active package headspace (**A**) and adsorbed in wild strawberries (**B**) packed in different 2-nonanone concentrations during storage at 10 and 22 °C.

temperature and on the quantity of 2-nonanone added to the active emitter ($p < 0.05$). Obviously, the higher the amount of compound is, the higher the concentration is in the fruit ($p < 0.05$). With respect to temperature, the sorption of the compound was higher at 10 °C, independently of the amount added. This effect could be explained by the higher condensability of the compound at low temperatures and a lower permeation rate out of the package.

Final 2-nonanone concentrations in strawberries increased with the amount of compound introduced, from 1 to 11 mg of 2-nonanone/kg at 22 °C and from 3 to 21 mg of 2-nonanone/kg fruit at 10 °C. Although the concentration increased as compared to the initial content (as expected), the content is far from toxic. In fact, the amount of 2-nonanone consumed in a 100 g serving would be ca. 1 million times below the LD_{50} critical value for mammalian toxicity.

Visual Appearance. Fungal infection by *Botrytis* growth on the surface tissue was visually estimated during the test, since this is the main cause of postharvest loss (*27*, *28*). In this study, incidence of fungal growth was only detected in samples without the antifungal compound held at room temperature; 10% of the fruit showed fungal decay. At 22 °C, the synergistic effect of high concentrations of CO_2 (*23*) and the presence of 2-nonanone inhibited the growth of *B. cinerea* at all of the antifungal concentrations tested. On the other hand, none of the packages stored at low temperature (10 °C) presented fungal decay. The effect of low temperature and CO_2 concentration (8−10%) were enough to produce a fungistatic effect, masking the antifungal effect of the 2-nonanone. Thus, for both of the temperatures assayed, the wild strawberry fruits packed in active packages remained rot-free during storage. This result was in agreement with that reported by Vaughn et al. (*14*), where starch-encapsulated (slow release) 2-nonanone reduced the *B. cinerea*-induced decay of raspberries and strawberries in enclosed containers stored at 10 °C.

ACKNOWLEDGMENT

We thank Georgina Hardinge for the English revision of the manuscript.

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Received for review October 1, 2006. Revised manuscript received January 15, 2007. Accepted January 22, 2007. This research was supported by the Spanish Ministry of Science and Technology (MCYT) (AGL2003-07326-C02-01).

JF062809M