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Optimization of an active package for wild strawberries based on the release of 2-nonanone

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A B S T R A C T

Active packaging is becoming in an emerging food technology to improve quality and safety of food products, commonly based on the retention or release of compounds which are beneficial for the product. In this work, an active packaging system based on the release of 2-nonanone has been optimized to increase the postharvest shelf life of fresh wild strawberries during the marketing stage. To avoid that excessive levels of this volatile could affect the berries' taste and cause consumer rejection of the product, a preliminary sensory analysis was carried out to determine the threshold value of 2-nonanone, 7.16 mg/kg fresh wild strawberries. Taking this threshold value into account, diverse quantities of 2-nonanone were tested to optimize the packaging parameters. Wild strawberry fruits were packaged in the active packages developed and their quality monitored during storage at 10 °C with exposure to light to simulate real-life conditions on supermarket shelves. The analyses of weight loss, SSC, gas composition and aroma volatiles provide evidences that exposure to the highest-tested 2-nonanone concentrations are an effective way of maintaining the quality of wild strawberries during distribution and sale without modifying their typical taste.

1. Introduction

Continuous efforts in fresh produce preservation, distribution and marketing are being made worldwide to supply consumers with high quality products. At present, appropriate postharvest techniques including controlled atmosphere (CA) and equilibrium modified atmosphere packaging (EMAP) combined with low temperature are commonly used for that purpose with excellent results. Nevertheless, novel preservation methods such as active packaging are being studied and developed to maintain product quality during a longer marketing period (Lee, Hwang, & Cho, 1998; Valverde et al., 2005).

Antimicrobial active packaging is one of the most promising food active packaging concepts for extending the shelf life of fresh produce. This technology prevents microbial growth on the product by means of interactions between the food and the packaging materials, or interactions with substances released by the package into the headspace. Among the active substances used in the design of active packages, compounds of natural origin such as several components of plant essential oils and food aromas are preferred (Hamilton-Kemp, McCracken, Loughrin, Andersen, & Hildebrand, 1992; Vaughn, Spencer, & Shasha, 1993; Wilson, Franklin, & Otto, 1987). Unwanted synthetic fungicides can be replaced by the incorporation of these natural agents within the package walls or in an independent sachet (Lee et al., 1998; Zivanovic, Chi, & Draughon, 2005). However, although most of these substances are GRAS, their use is often limited because of their impact on the organoleptic characteristics of the product.

Wild strawberries (*Fragaria vesca* L.) are berries with a high added value, but are very complex to market fresh due to their rapid postharvest deterioration. Although efficient conservation technologies such as controlled atmosphere storage and modified atmosphere packaging have been developed for delaying that fast decay (Almenar, Del-Valle, Hernández-Muñoz, et al., 2007; Almenar, Hernández-Muñoz, Lagarón, Catalá, & Gavara, 2006), an antimicrobial active packaging based on the combination of equilibrium modified atmosphere and 2-nonanone, a naturally occurring fruit aroma volatile, has showed higher effectiveness (Almenar, Del-Valle, Catalá, & Gavara, 2007). Therefore, a possible commercial application of this package could increase shelf life and bring greater export opportunities.

Although 2-nonanone has been proved to delay fungal growth at high concentrations, the exogenous addition of this volatile compound could affect the natural organoleptic properties of the fruit by causing a strange odor or by unbalancing the aroma. To avoid safety and taste problems, which are both very important in

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consumer choice, a study of the effect of the concentration of the antimicrobial agent on the properties of the fruit is needed, including sensory tests.

As a result, this study focuses on the optimization of an antifungal active package system based on the release of 2-nonanone. A previous sensory evaluation was carried out to estimate the consumer threshold level for 2-nonanone in strawberries. Wild strawberries were then stored for 4 days at 10 °C in packages with varying initial amounts of the antifungal agent. The effect of the 2-nonanone on the berries was evaluated through several quality parameters and by sensory evaluation.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

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

2.1.2. Packaging material

125-mL polypropylene/ethylene vinyl alcohol/polypropylene (PP/EVOH/PP) cups (300 μm thick, 4.7 cm high and 7.6 cm opening diameter) supplied by Huhtamaki (Nules, Castellón, Spain) were used as containers and 50-μm polyethylene terephthalate/polypropylene (PET/PP) multilayer films with 3 microperforations (100 μm diameter) supplied by Amcor Flexible (Bristol, U.K.) were used as lids. A previous study had demonstrated that this package provided an adequate equilibrium atmosphere for wild strawberries (Almenar, Del-Valle, Hernández-Muñoz, et al., 2007). Metallocene polypropylene (mPE) sachets containing alumine F-1 80/100 mesh (Supelco Inc., Bellefonte, PA, USA) (0.1 g per sachet) impregnated with 0, 0.1, 0.5, 1, 2 and 3 μL 2-nonanone (Sigma–Aldrich Chemie, Steinheim, Germany) were used as the active emitter. mPE was selected as the film material for manufacturing the active bags due to its high permeability to organic compounds (Cava, Catala, Gavara, & Lagaron, 2005). The alumina and the liquid agent were mixed with a vortex mixer in a closed vial. The sachets were then filled and heat-sealed. The sachets with the active compound were adhered to the inside of the lid before heat-sealing the cups.

2.2. Methods

20 wild strawberry fruits were selected at random and placed in PP/EVOH/PP cups. Each cup was sealed with the lid material after adhering the 2-nonanone emitter system to the lid. Samples were stored at 10 °C and 77% RH and tested at days 0, 2 and 4 of storage. Packages without the volatile compound were also stored and sampled for comparison.

6 cups per 2-nonanone amount and storage time were evaluated; three of them (three replicates) were used to measure headspace composition, 2-nonanone concentration and fungal decay and the rest were mixed and blended to obtain a purée. The purée was then subjected to different tests to measure the other quality parameters, using three replicates from each treatment, and the mean values were calculated. Before beginning the daily measurements, every package was weighed. Sixty wild strawberry fruits were chosen at random for the evaluation of quality characteristics on day 0.

2.2.1. Weight losses

The net ($W_{\mathrm{strawberry}}$) and gross (W_0) weights of each package were recorded at day 0 with a Voyager analytical balance (Ohaus, Suiza) and the gross weights were recorded over the storage time (W_{t}). The weight loss percentage was obtained as follows:

$$\text{Weight loss } (\%) = \frac{(W_0 - W_{\mathrm{t}}) \times 100}{W_{\mathrm{strawberry}}}$$

2.2.2. Headspace composition

The CO_2 and O_2 contents of each package were 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 GC (Agilent Technology, Barcelona, Spain) equipped with a thermal conductivity detector (TCD) and a Chromosorb 102 column (Restek, Teknokroma, Barcelona, Spain). The gas carrier was helium. 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).

The 2-nonanone concentration inside each package was also analyzed. 100 μL headspace gas was injected into a Hewlett–Packard 5890 series II GC (Agilent Technology, Barcelona, Spain) equipped with a flame ionization detector (FID) and a Rtx-1301 column (0.50 μm × 0.53 mm, 30 m, Restek, Teknokroma, Barcelona, Spain). The gas carrier was helium. The oven temperature was initially 40 °C for 5 min and was then raised to 200 °C at 5 °C/min and maintained for 2 min. The injector and detector temperatures were 240 °C. Quantification was performed by comparison with GC calibration standards previously obtained by injecting known amounts of 2-nonanone. During sampling, three different packages per 2-nonanone concentration were analyzed.

The content of several volatile organic compounds was monitored during storage to check for effects on the aroma profile caused by the release of 2-nonanone. The selected compounds were 3 fermentative metabolites (acetaldehyde, ethyl acetate and ethanol) and 9 strawberry aroma components (methyl butyrate, ethyl butyrate, ethyl hexanoate, hexanal, 2-heptanone, 2-nonanone, hexanoic acid, 1-hexanol, *trans*-2-hexen-1-ol (hexenol), β-citronellol). All these compounds were identified by GC–MS and monitored by GC–FID using the procedure described below.

2.5 g samples of strawberry purée were placed in 10 mL vials, crimp-sealed and frozen at −20 °C. For GC analysis, the 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 micro-extraction (SPME) using a 65 μm PDMS/DVB SPME fiber (Supelco Inc., Barcelona, Spain). The fiber was exposed to the vial headspace during 20 min and the trapped volatiles were immediately desorbed (for 5 min) at the splitless injector port of a GC Hewlett–Packard 5890 series II. The samples were analyzed using the same chromatographic conditions described above for 2-nonanone. Three vials by treatment were analyzed. Quantification was performed by comparison with prior GC calibration using the addition method: 6 g strawberry purée samples were supplemented with increasing known amounts of the volatile compounds and were analyzed following the procedure already described. Three vials per treatment were analyzed.

2.2.3. Titratable acidity

Berries from packages with the same 2-nonanone concentration were homogenized in a Moulinex blender, divided in three parts (to serve as replicates) and immediately analyzed. 6 g of the purée were diluted with 100 mL distilled water and filtered to remove pulp. The acidity expressed as mg citric acid/100 mL juice was measured by titration with 0.1 N NaOH and the end-point of pH 8.1.

2.2.4. Soluble solids content

The total soluble solids (TSS) content of the strawberry purée was measured with an Atago RX-1000 digital refractometer (Atago Co., Ltd., Tokyo, Japan). The results were expressed as °Brix.

2.2.5. Fungal decay

The presence of *Botrytis cinerea* was visually estimated in each individual fruit right 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*.

2.2.6. Sensory evaluation

Two tests were carried out. The first, a triangular test (ISO, 2004), was performed to detect the amount of 2-nonanone that could be present in strawberries without being perceived sensorially (threshold level). The samples were prepared for testing by adding 0, 1, 3, 5 and 10 μL of 2-nonanone to 1 kg of strawberry purée. The final 2-nonanone concentrations were 3, 3.83, 5.50, 7.16 and 11.32 mg 2-nonanone/kg strawberry, respectively. Three series of three samples each (two controls and a sample with 2-nonanone) were offered to an untrained panel of 35 members (aged 20–40 years). After tasting each series once in accordance with the method, every panelist was asked to distinguish which of the three samples presented simultaneously was the different one. The strawberry purée was presented in a small plastic glass, from which the judges sampled it using a coffee spoon. A three digit random number code was placed on the glass containing each sample. The position of the test samples in each series was randomized.

A second sensory test, a ranking test (ISO, 1988a), was carried out to determine flavor, odor, acidity and appearance differences in berries packed with increasing amounts of 2-nonanone. Each panelist (out of a total of forty one) was provided with 5 packages (0.1, 0.5, 1, 2 and 3 μL of 2-nonanone), which were presented with random number codes in a randomized order of presentation. After opening the packages, the above-mentioned sensory attributes were evaluated.

Both tests were carried out in individual cabins as described in ISO 4120:2004 (ISO, 2004), and were performed in accordance with European cooperation for Accreditation of Laboratories criteria (ISO, 1988b). Data acquisition was conducted using a Compusense® *five* release 4.6 (Compusense Inc., Guelph, ON, Canada).

2.2.7. Statistical analysis

The StatGraphics Plus program version 2.1 (Statistical Graphics Corp., USA) was used for the analysis of variance (ANOVA) and to test significant differences between means with $p < 0.05$.

3. Results and discussion

3.1. 2-Nonanone threshold level

In order to avoid modifications in the strawberry taste caused by the active package which could lead to consumer rejection, a triangular test was carried out to identify the minimum concentration of 2-nonanone in strawberries which could be detected through fruit consumption. Results from this test showed that purées containing 3.83, 5.50 and 7.16 mg 2-nonanone/kg strawberry were not distinguished from the control sample, which contained 3 mg/kg, whereas the strawberry purée containing 11.32 mg 2-nonanone/kg strawberry was recognized as different ($p < 0.05$). Consequently, the threshold level is somewhere within the interval of 7.16–11.320 μg 2-nonanone/kg strawberry. Therefore, to optimize the active package, the lower of these values was selected as the maximum concentration of the active agent which could be present in packaged fruits during storage. In a preliminary test, fruits were exposed to active packages containing 1, 2.5, 5 and 10 μL of the antifungal agent and the results showed that after 24 h, the samples with 5 and 10 μL largely exceeded this limit, so the study was limited to packages containing up to 3 μL of 2-nonanone.

Fig. 1 shows the evolution of 2-nonanone concentrations in wild strawberries during storage at 10 °C in active packages containing different amounts of the active agent. It also shows the above-mentioned threshold level of 2-nonanone. Obviously, the concentration increases with the amount of 2-nonanone added to the packaging system. Berries packed with 3 μL of 2-nonanone reached a concentration of this volatile that was slightly above the lower threshold level, nevertheless, the panelist did not perceive the presence of the compound in the preference test results reported below. The 2-nonanone content in the packages containing the lowest amounts of the compound had even decreased at the end of the storage. This result could be a consequence of loss of the compound due to permeation through the porous lid of the container. Therefore, all the 2-nonanone concentrations tested during this study can be considered acceptable. Moreover, these low concentrations of 2-nonanone were 1000- to 100-fold lower than its LD_{50} (3200 mg/kg) (NIOSH, 1979).

3.2. Evolution of 2-nonanone in headspace

Table 1 shows the levels of 2-nonanone reached in the headspace of the packages after 2 and 4 days of storage at 10 °C. As can be seen, the levels of this volatile were totally dependent on the initial volatile inserted ($p < 0.05$), besides remaining fairly constant during storage. Concentrations of between 0.012 and 0.075 μL 2-nonanone/L air were reached inside the packages after 4 days of storage. Since the active package was designed to be capable of releasing 2-nonanone slowly over time, it could be considered that the headspace concentration is in a stationary balance where what is lost through sorption into the product or the package or by permeation into the environment is replaced by delivery from the sachet.

3.3. Weight loss

Weigh loss in wild strawberries is higher than in other fresh products and is one of the most important causes of quality deterioration. This quality parameter has been reported as approximately 6% before marketability is impaired (Robinson, Browne, & Burton, 1975). As seen in Fig. 2, this value was not reached by any of the

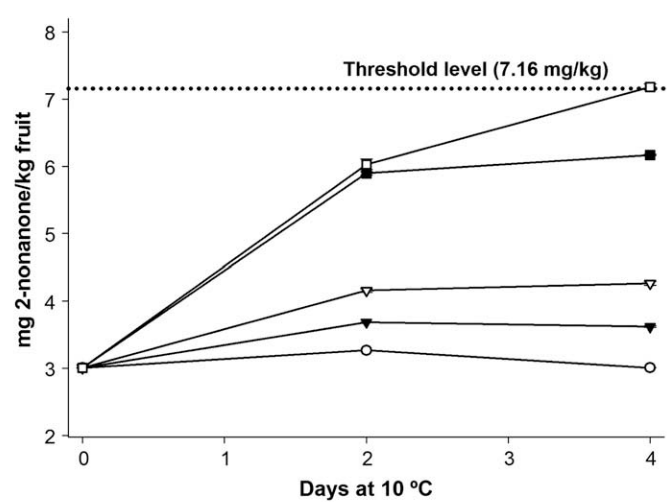

Fig. 1. Threshold level perceived in sensory evaluation and amounts of 2-nonanone absorbed by wild strawberries (mg 2-nonanone/kg) during storage at 10 °C and different amounts of 2-nonanone: (○), 0.1 μL; (▼), 0.5 μL; (▽), 1 μL; (■), 2 μL; and (□), 3 μL.

Table 1
2-Nonanone concentrations in package headspace after 2 and 4 days of storage at 10 °C.

2-Nonanone (μL)	μL 2-nonanone/mL air	
	Day 2	Day 4
0.1	$0.014^{a\alpha}$	$0.012^{a\alpha}$
0.5	$0.024^{b\alpha}$	$0.021^{b\alpha}$
1	$0.027^{b\alpha}$	$0.029^{c\alpha}$
2	$0.063^{c\alpha}$	$0.059^{d\alpha}$
3	$0.066^{c\alpha}$	$0.075^{e\alpha}$

Superscript letters (a–e) mean significant differences ($p < 0.05$) among 2-nonanone concentrations reached in package headspaces on the same day.
Superscript letter (α) means no significant difference ($p < 0.05$) in 2-nonanone concentration reached during the storage period in each packaging system.

packages after 4 days of storage at 10 °C since all berries were packaged in EMA, which allowed the respiration rate to slow down and consequently reduced weight loss. Moreover, the high relative humidity generated inside the packages was also responsible for the delay in drying and hardening of the fruit. These results were expected since previous studies (data not shown) at 10 °C had shown that the weight loss of wild strawberries packaged with the same microperforated films was 65-fold lower than that found in berries without packaging after 6 days of storage.

As expected, the weight loss of wild strawberries increased as a function of storage time for all packages. Among the samples analyzed, after 4 days of storage at 10 °C a higher water loss was observed for packages without 2-nonanone (0.43%), since significant reductions ($p < 0.05$) were obtained with the addition of the volatile (up to 16%). No significant differences were found on comparing different active packages, except for those with the lowest amount of volatile (0.1 μL). However, a clear trend towards weight loss reduction was observed as the 2-nonanone increased. The differences among treatments were related exclusively to the exogenous addition of 2-nonanone, since weight loss is dependent on the water vapour permeability of the packaging film and the relative humidity of the storage atmosphere and both can be considered identical for all packages.

3.4. Headspace composition

To reduce the high respiration rate of wild strawberries, equilibrium modified atmosphere packaging technology is commonly used. A suitable atmosphere composition can reduce the respiration rate of the fruit and the growth of fungi with minimal alteration of organoleptic properties.

Fig. 3 shows the headspace composition reached in each treatment at the end of the storage period. As can be seen, the initial atmosphere composition inside the packages (air) was modified during storage due to the respiration of the berries and the permeability of the packaging material. Nonetheless, the evolution of CO_2 and O_2 concentrations depended significantly on the treatment ($p < 0.05$). Packages without 2-nonanone showed the highest CO_2 and lowest O_2 levels (13.7 and 11.7%, respectively). The addition of 2-nonanone significantly affected the composition of the headspace, a result which could be related to an apparent reduction in the strawberries' respiration rate. This effect increases with the amount of 2-nonanone added. Nevertheless, no significant differences were observed among samples with a volatile content above 1 μL.

3.5. Soluble solid content

The soluble solid content (SSC) of wild strawberries was measured to determine the effect of 2-nonanone on the stage of maturity of the fruits. As can be seen in Table 2, the SSC of berries from all packages decreased with the storage time from an initial value of 11.97 °Brix. However, significant differences ($p < 0.05$) were observed among packages. At the end of the storage at 10 °C, control packages reached 10.24 °Brix while 2-nonanone-containing packages ranged between 11.07 and 11.30 °Brix. The higher SSC depletion from packages without 2-nonanone was in agreement with their higher CO_2 levels (Fig. 3), given the well-known relationship between the sugar content and respiration rate of harvested fruit, which consume sugars to maintain their physiological activity. Regarding to the different active packages, lower SSC depletion was observed when higher 2-nonanone amounts were present inside the package (Table 1). Therefore, it may be supposed that 2-nonanone increases wild strawberry shelf life by reducing the respiration rate and SSC depletion, two important quality parameters of fresh produce.

3.6. Titratable acidity

Titratable acidity (TA) plays an important role in cell pH regulation, anthocyanin stability and fruit flavor. Citric acid is the major organic acid found in strawberry fruit (Kim & Moon, 1993), accounting for 88% of the total organic acids in ripe fruit. As can

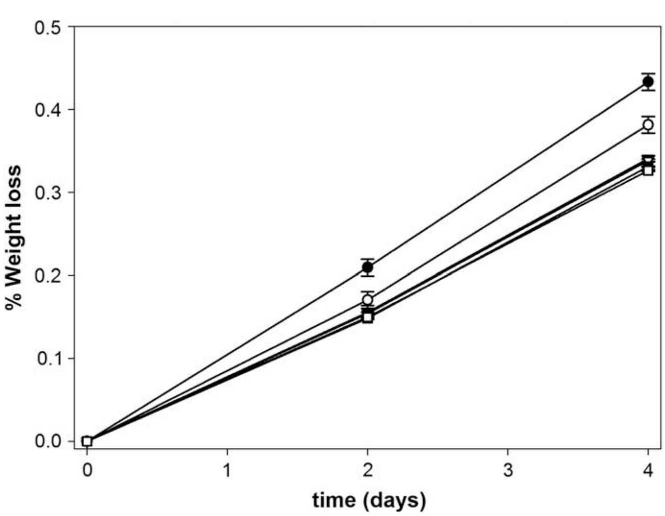

Fig. 2. Percentage of weight loss over 4 days at 10 °C in strawberries stored in active packages containing different amounts of 2-nonanone: (●), 0 μL; (○), 0.1 μL; (▼), 0.5 μL; (▽), 1 μL; (■), 2 μL; and (□), 3 μL.

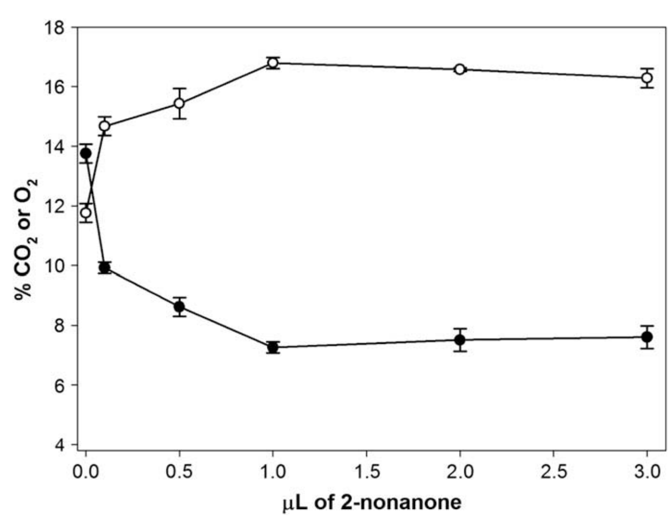

Fig. 3. CO_2 (●) and O_2 (○) contents after 4 days at 10 °C in the headspace of packages containing strawberries and different amounts of 2-nonanone.

Table 2
Evolution of soluble solid content (°Brix) and titratable acidity (mg citric acid/100 mL juice) of wild strawberries packaged with different amounts of 2-nonanone and stored at 10 °C for 2 and 4 days.

2-Nonanone (μL)	Soluble solid content	Titratable acidity
Day 0		
0	11.97^{α}	0.96^{α}
Day 2		
0	$11.24^{a\beta}$	$0.75^{a\beta}$
0.1	$11.23^{a\beta}$	$0.71^{b\beta}$
0.5	$11.57^{b\beta}$	$0.64^{c\beta}$
1	$11.63^{c\beta}$	$0.64^{c\beta}$
2	$11.76^{d\beta}$	$0.62^{a\beta}$
3	$11.77^{d\beta}$	$0.61^{a\beta}$
Day 4		
0	$10.24^{a\gamma}$	$0.73^{a\gamma}$
0.1	$11.07^{b\gamma}$	$0.70^{b\gamma}$
0.5	$11.12^{bc\gamma}$	$0.68^{c\gamma}$
1	$11.17^{c\gamma}$	$0.65^{d\gamma}$
2	$11.23^{d\gamma}$	$0.59^{e\gamma}$
3	$11.30^{e\gamma}$	$0.58^{e\gamma}$

Superscript letters (a–e) indicate significant differences ($p < 0.05$) between °Brix or mg citric acid/100 mL juice reached in strawberries packaged with different quantities of 2-nonanone.
Superscript letters (α–γ) indicate significant differences ($p < 0.05$) between °Brix or mg citric acid/100 mL juice at 0, 2 and 4 days of treatment for each quantity of 2-nonanone.

been seen in Table 2, the initial values of this acid (0.96 mg citric acid/100 mL strawberry juice) decreased as a function of storage time. This table also shows that the concentration of 2-nonanone significantly affects ($p < 0.05$) the evolution of acidity in these fruits. The TA depletion was expected since ripening is characterized by a decrease in citric acid, moving towards a minimum at the overripe storage stage (Green, 1971). On comparing treatments, the changes in TA were clearly related to the concentrations of 2-nonanone reached inside the packages (Table 1). The higher the 2-nonanone concentration, the higher the citric acid depletion. Thus, at the end of the storage period, berries from control packages reached 0.73 mg citric acid/100 mL juice, while those from packages with 2-nonanone ranged from 0.70 to 0.58 mg citric acid/100 mL juice.

García, Medina, and Olías (1998) and Picón, Martinez-Javega, Cuquerella, Del Rio, and Navarro (1993) reported the lowest acidity in cultivated strawberries packed in the lowest O_2 content. This research is in agreement, as it shows that lower acidity is reached when low 2-nonanone treatments are used, that is to say, lower O_2 and higher CO_2 concentrations. According to these results, the lower CO_2 accumulation reached inside packages with higher 2-nonanone concentrations could be related to higher berry acidification, since it could involve the absorption of this gas by the fruit and its subsequent acidification.

Since both a high sugar content and a relatively high acidity content are required for good cultivated strawberry quality (Kader, 1990), active packaging with 2-nonanone could indicate a better way to assure high quality in wild strawberries as compared with EMA packaging. For an acceptable flavor, maximum 0.8% TA and minimum 7% SSC have been recommended (Mitcham, Crisosto, & Kader, 1996). As can be seen in Table 2, berries from all the packages fitted within the recommended values.

3.7. Volatiles

One of the most important processes occurring during fruit ripening is the increase in volatiles which contribute to fruit aroma and flavor. In wild strawberries, major volatiles identified include hexanoic acid, *trans*-2-hexen-1-ol, methyl butanoate, 1-hexanol, ethyl butanoate and 2-heptanone (Pyysalo, Honkanen, & Hirvi, 1979). Additionally, other volatiles such as fermentative metabolites can also have an impact on berry flavor. In this study, the concentration–effect relationship of 2-nonanone with those volatiles was analyzed in berries at 0 and 4 days after packaging and storing at 10 °C. As shown in Table 3, the levels of acetaldehyde, ethanol and ethyl acetaldehyde at harvest (2, 54 and 2.8 mg/kg, respectively) increased greatly ($p < 0.05$) in response to high CO_2 and/or low O_2 levels found in EMA packaging after 4 days of storage (Fig. 3). Similar off-flavor accumulations were also reported by Ke, Goldstein, O'Mahony, and Kader (1991) and Larsen and Watkins (1995) for cultivated strawberries stored in modified atmospheres. However, different increases were observed ($p < 0.05$) depending on the 2-nonanone inserted. Ethanol and ethyl acetate concentrations increased but acetaldehyde decreased as the volatile concentration increased. Different off-flavor evolutions could be related to the different atmospheres reached inside the packages. The observed acetaldehyde depletion was in agreement with results obtained for wild (Almenar, Del-Valle, Catalá, et al., 2007; Almenar, Del-Valle, Hernández-Muñoz, et al., 2007) and cultivated (Pesis, 2005) strawberries stored in higher CO_2 concentrations.

Additionally, the effects of both 2-nonanone and EMA packaging on the main aroma compounds of wild strawberries were studied. As can be seen in Table 4, packaging in EMA conditions had a profound effect on the volatiles profile of wild strawberries. At harvest, this profile showed higher concentrations of hexanal, followed by hexanoic acid and ethyl butanoate. 1-Hexanol and *trans*-2-hexen-1-ol had similar concentrations. Lower concentrations were found for ethyl hexanoate, methyl butanoate, β-citronellol and 2-heptanone. However, this profile was totally modified due to the increase in the concentration of esters and the decrease in the concentration of carbonyl compounds and acids. This is in agreement with Larsen and Watkins (1995), who observed an increase in ethyl esters in cultivated strawberries exposed to high CO_2 concentrations. These compounds are related to the fruity aromatic note of these fruits (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2003; Ulrich, Hoberg, Rapp, & Kecke, 1997). Also, Forney, Kalt, McDonald, and Jordan (1998) reported that the level of volatile esters in cultivated strawberries increased during ripening on and off the plant.

In addition to packaging, 2-nonanone concentration also affected the production of aroma compounds in wild strawberries. After 4 days at 10 °C, ethyl hexanoate was the ester with the highest increase caused by 2-nonanone. According to Forney, Kalt, and Jordan (2000), this volatile has been found to be the most consistent high intensity aroma in different strawberry cultivars sampled. Therefore, exposure to 2-nonanone could be supposed to improve wild strawberry aroma. Concentrations of C_6 alcohols and C_6

Table 3
Changes in fermentative compound concentrations in wild strawberries packed with different quantities of 2-nonanone, after 4 days of storage at 10 °C.

2-Nonanone (μL)	mg volatile/kg FW		
	Acetaldehyde	Ethanol	Ethyl acetate
Day 0			
0	2.0^{α}	54^{α}	2.8^{α}
Day 4			
0	$15.2^{a\beta}$	$391^{a\beta}$	$11.5^{a\beta}$
0.1	$15.5^{a\beta}$	$407^{b\beta}$	$12.1^{a\beta}$
0.5	$13.5^{b\beta}$	$479^{c\beta}$	$11.6^{a\beta}$
1	$12.8^{b\beta}$	$664^{d\beta}$	$15.6^{b\beta}$
2	$11.9^{c\beta}$	$660^{d\beta}$	$15.0^{b\beta}$
3	$11.5^{c\beta}$	$656^{d\beta}$	$15.3^{b\beta}$

Superscript letters (α, β) indicate significant differences ($p < 0.05$) among concentrations of fermentative metabolites caused by packaging.
Superscript letters (a–d) Indicate significant differences ($p < 0.05$) among concentrations of fermentative metabolites due to 2-nonanone quantity.

Table 4
Changes in the concentrations (mg/kg FW) of the main volatile compounds of wild strawberries packed with different quantities of 2-nonanone after 4 days of storage at 10 °C.

2-Nonanone (μL)	Concentration of aroma compounds (mg/kg fruit)								
	Esters			Alcohols			Carbonyl		Acid
	MeBu	EtBu	EtHx	HxOH	HxeOH	Citr	HxO	HpO	HxAc
At harvest	0.20^{α}	0.61^{α}	0.44^{α}	0.53^{α}	0.58^{α}	0.18^{α}	13.1^{α}	0.07^{α}	0.72^{α}
0	$0.25^{\beta a}$	$0.78^{\beta a}$	$8.12^{\beta a}$	$0.54^{\alpha a}$	$0.60^{\alpha a}$	$0.23^{\alpha a}$	$0.31^{\beta a}$	$0.04^{\beta a}$	$0.64^{\beta a}$
0.1	0.24^{a}	0.37^{b}	37.8^{b}	0.50^{a}	0.55^{b}	0.35^{b}	0.22^{b}	0.03^{a}	0.62^{a}
0.5	0.23^{a}	0.34^{b}	39.2^{c}	0.50^{a}	0.47^{c}	0.58^{c}	0.17^{c}	0.04^{a}	2.03^{b}
1	0.26^{a}	0.28^{d}	40.0^{c}	0.50^{a}	0.42^{d}	0.76^{d}	0.13^{d}	0.04^{a}	3.29^{c}
2	0.27^{a}	0.25^{b}	40.1^{c}	0.50^{a}	0.40^{d}	1.24^{e}	0.02^{e}	0.03^{a}	7.19^{d}
3	0.28^{a}	0.23^{b}	40.9^{c}	0.50^{a}	0.39^{d}	1.46^{f}	0.01^{e}	0.03^{a}	9.60^{e}

MeBu, methyl butanoate; EtBu, ethyl butanoate; EtHx, ethyl hexanoate; HxOH, 1-hexanol; HxeOH, *trans*-2-hexen-1-ol; Citr, β-citronellol; HxO, hexanal; HpO, 2-heptanone; HxAc, hexanoic acid.
Superscript letters (a–d) indicate significant differences ($p < 0.05$) among concentrations of aroma compounds of wild strawberries caused by the amount of 2-nonanone.
Superscript letters (α, β) indicate significant differences ($p < 0.05$) among concentrations of aroma compounds of wild strawberries caused by packaging.

aldehydes, a grassy or herbaceous aromatic note, showed a decrease or no change in concentration depending on the compound. Other alcohols such as β-citronellol showed an increase which was totally dependent on the amount of 2-nonanone added. Hexanoic acid was the compound most affected by 2-nonanone concentration. The concentration of this acid was 3-, 5-, 10- and 15-fold higher when berries were exposed to 0.5, 1, 2 and 3 μL of 2-nonanone, respectively.

3.8. Sensory evaluation

The preference test carried out showed no significant differences among the different packages tested. Therefore, no significant differences in the general appearance or taste of wild strawberries packed with amounts of 2-nonanone ranging from 0.1 to 3 μL were observed after 4 days of storage. These results were expected because in all cases the assayed amounts of volatile were lower than the threshold level. However, at the higher 2-nonanone quantities tested, a slight odor of this volatile was perceived immediately on opening.

3.9. Fungal decay

Rot development is the best indicator of wild berry storage life, besides being the main reason for consumers' discarding fruit. In this study, berries treated with 2-nonanone had no visible fungi and no fruit damage. Nevertheless, 10% of fruits in the control samples presented evidence of fungal development. Vaughn et al. (1993) observed fungal growth completely inhibited without causing necrotic tissue in cultivated strawberries exposed to 2-nonanone in the range of about 0.02 to about 0.20 μL/mL air. In addition, it could be suggested that the higher acetaldehyde production by the fruit in response to 2-nonanone insertion assists in controlling *Botrytis* decay, since it has been suggested that acetaldehyde disrupts *B. cinerea* cell membranes as a first step towards inhibition of fungus activity induced by leakage of electrolytes, sugars and amino acids from the fungus (Avissar, Droby, & Pesis, 1990).

4. Conclusions

It is evident from this study that equilibrium modified atmosphere packaging and the release of 2-nonanone are complementary technologies that are capable of improving berry shelf life. The addition of 2-nonanone at low concentrations does not result in sensory deterioration but reduces the incidence of fungal growth and delays senescence by decreasing the rate of postharvest physiological activity.

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