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Controlled Atmosphere Storage of Wild Strawberry Fruit (*Fragaria vesca* L.)

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Controlled atmosphere storage technology to extend the shelf life of "Reina de los Valles" wild strawberry fruit (*Fragaria vesca* L.) was studied. Fruits were stored at 3 °C for three weeks in different atmosphere compositions: 0.05% CO_2/21% O_2 (air), 3% CO_2/18% O_2, 6% CO_2/15% O_2, 10% CO_2/11% O_2, and 15% CO_2/6% O_2. The effect of gas composition on soluble solids content, titrable acidity, pH, off-flavor, aroma volatiles, and consumer preference was monitored. The result showed that the 10% CO_2/11% O_2 combination can efficiently prolong the shelf life of wild strawberries by maintaining the quality parameters within acceptable values, through inhibiting the development of *Botrytis cinerea*, without significantly modifying consumer acceptance.

KEYWORDS: Wild strawberry (*Fragaria vesca* L.); *Botrytis cinerea*; quality parameters; postharvest self-life; carbon dioxide; oxygen.

INTRODUCTION

Wild strawberries are a product that is highly appreciated by consumers. Their natural aspect, color, nutritional values, and high natural antioxidant compound content are their most attractive characteristics. A high percentage of these fruits is sold as a frozen product which is used in the manufacture of cakes, ice creams, or milk desserts. Despite their high price, there is an increasing demand for fresh berries and, consequently, a need to increase their distribution ratio and shelf life.

The postharvest conservation of wild strawberries is very complex. A very fast metabolism leads to rapid senescence, which may be controlled by the use of low-temperature (prompt cooling to near 0 °C) and high-humidity storage (*1*). Nevertheless, that is not sufficient to reduce fungal decay. As the surface of these berries is extremely sensitive, external washing to eliminate soil particles and, more critically, to reduce microbial contamination is not recommended. Thus, a significant proportion of fresh strawberries is lost every year (mainly through *Botrytis cinerea*) (*2*). Methods such as fumigation are being avoided because of consumer awareness of food safety and nutritional value. Therefore, other technologies such as storage in a suitable atmosphere at low temperature seem to be a feasible option.

The use of a CO_2-enriched atmosphere is a widespread postharvest practice to control fungal decay in fresh fruit and vegetable products. Several studies have reported that controlled atmosphere storage of different cultivated strawberry varieties may increase their shelf life by delaying both senescence and fungal decay. These effects are associated with the reduction of respiration and ethylene production rates (*3*−*5*). Fruits exposed to high levels of CO_2 during cool storage showed firmness enhancement (*6*, *7*) and lower susceptibility to decay (*3*). However, although combinations of high-CO_2 and low-O_2 atmospheres improve most strawberry quality parameters, the generation of off-flavor compounds (ethanol and ethyl acetate) increases, producing an adverse sensory effect (*8*). High-CO_2 and high-O_2 atmospheres do not relieve these off-flavor problems and appear to induce a synergistic effect that even increases the production of fermentative metabolites (*8*, *9*).

In this study, wild strawberries were stored in different controlled atmosphere compositions containing from 0.05 to 15% CO_2 and from 21 to 6% O_2 at 3 °C. The effect of CO_2 and O_2 combinations on the chemical composition, physical quality parameters, mold growth, and sensory quality of wild strawberries was monitored over three weeks.

MATERIALS AND METHODS

Materials. *Reagents*. Acetaldehyde, ethyl acetate, ethanol, methyl butyrate, ethyl butyrate, ethyl hexanoate, hexanal, 2-heptanone, 2-nonanone, 2,5-dimethyl-4-hydroxy-3(2h)-furanone (furanone), acetic acid, hexanoic acid, methanol, benzyl alcohol, 1-hexanol, *cis*-3-hexen-1-ol (hexenol), and β-citronellol) were purchased from Sigma-Aldrich and used in gas chromatography for compound identification and calibration.

Plant Material. Wild strawberries (*Fragaria 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, nonuniform, unripe, or overripe fruits were eliminated, and the selected fruits were stored for at least 2 h at 3 °C to ensure equilibrium.

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Controlled Atmosphere Storage. Eight open trays containing 250 g of wild strawberries were placed in each of the 10-L chambers, which were equipped with inlet−outlet ports, to maintain the product within the desired controlled atmosphere environments for three weeks at 3 °C. Precision needle valves maintained a constant flowrate of about 20 mL/min. The selected gases were 0.05% CO_2/21% O_2 (air), 3% CO_2/18% O_2, 6% CO_2/15% O_2, 10% CO_2/11% O_2, and 15% CO_2/6% O_2 (balanced with N_2). The gas mixtures were humidified in washing bottles. The compositions of the headspace gas mixtures were verified each day by taking 100-μL gas samples and analyzing them in a gas chromatograph equipped with a thermal conductivity detector (*10*).

The effect of the controlled atmosphere storage on the fruits was monitored during storage. At each assessment time, a 250-g sample of berries was taken from each container, divided into three lots, and immediately analyzed.

Methods. *Titrable Acidity and pH.* A 250-g sample of berries was taken from each container and homogenized in a Moulinex blender, divided into three lots, and immediately analyzed. A 6-g sample of the 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.

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

Volatile Compounds. The content of several volatile organic compounds was monitored during storage in the different controlled atmosphere conditions. The selected compounds included three fermentative metabolites (acetaldehyde, ethyl acetate, and ethanol) and 17 typical strawberry flavor compounds (methyl butyrate, ethyl butyrate, ethyl hexanoate, hexanal, 2-heptanone, 2-nonanone, 2,5-dimethyl-4-hydroxy-3(2h)-furanone (furanone), acetic acid, hexanoic acid, methanol, benzyl alcohol, 1-hexanol, *cis*-3-hexen-1-ol (hexenol), β-citronellol). All these compounds were identified by GC-MS and monitored by GC-FID using the procedure described below.

Each 2.5-g sample of strawberry purée was placed in a 10-mL vial, 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 micro-extraction (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, Barcelona, Spain) equipped with FID and a Rtx-1301 column (0.50 μm × 0.53 mm × 30 m, Restek, Teknokroma, Barcelona, Spain). The oven temperature was initially 40 °C for 5 min; it was then increased 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. Previous compound identification was performed by GC-MS using the same column and chromatographic conditions. GC-MS and GC-FID peaks were correlated using the pure compounds. Quantification was performed after calibrating the GC-SPME system by the addition method. Known amounts of the volatile compounds were added to 6-g strawberry puree samples and were analyzed following the procedure already described.

Fungal Decay. The presence of molds was visually estimated in each individual fruit immediately after opening the chambers. A magnifying lens (4×) was used for this purpose. Wild strawberry fruits showing surface mycelial development were considered decayed. The results were expressed as percentage of fruits infected by *Botrytis*.

Sensory Evaluation. A triangular test (*11*) was used to check for sensory differences between strawberries stored in air and those maintained in the 15% CO_2 atmosphere. The tests were carried out at 10 days of storage due to the possible presence of fungal growth in strawberries maintained in air at a later stage. The sensory analysis was performed by an untrained panel of 35 members (aged 20 to 40 years) who were asked to distinguish between samples considering both overall appearance and taste. In accordance with the method, every panelist was asked to distinguish which of the three strawberries presented simultaneously was different. The berries were presented in a small tray from which the judges sampled them by hand. A code with a three-digit random number was placed next to each sample. The position of the test samples was randomized.

This test was carried out in individual cabins, as described in ISO 4120:2004 (*11*), and was performed in accordance with European cooperation for Accreditation of Laboratories criteria ISO 8589:1988 (*12*). Data acquisition was obtained using Compusense *five*, release 4.6, (Compusense Inc.: Guelph, ON, Canada).

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

Table 1. Effect of Different CO_2/O_2 Atmospheres on Soluble Solids Content (°Brix), Titrable Acidity (mg citric acid/100 mL juice) and pH Values of Wild Strawberries Stored for Three Weeks at 3 °C[a,b]

day	controlled atmosphere composition	soluble solids content	titrable acidity	pH
0		11.33 **A**	0.996 **A**	3.80 **A**
20	air	9.23 **a B**	0.751 **a B**	3.83 **a B**
	3% CO_2/18% O_2	9.65 **b B**	0.732 **b B**	4.00 **b B**
	6% CO_2/15% O_2	10.07 **c B**	0.684 **c B**	4.07 **c B**
	10% CO_2/11% O_2	10.33 **d B**	0.613 **d B**	4.11 **d B**
	15% CO_2/6% O_2	10.47 **e B**	0.590 **e B**	4.10 **e B**

[a] **A** and **B** mean significant differences between initial and final quality parameter values of wild strawberries stored in different CO_2/O_2 atmospheres. [b] **a**, **b**, **c**, **d**, **and e** mean significant differences among final quality parameter values of wild strawberries stored in different CO_2/O_2 atmospheres.

RESULTS AND DISCUSSION

Soluble Solids Content (SSC). The soluble solids content, pH, and acidity values as a function of time and atmospheric composition are presented in **Table 1**. As expected, the length of exposure produced a significant reduction in the soluble solid content of the berries as a consequence of their postharvest metabolism. At the end of the storage period, the product SSC had evolved from the initial 11.33 °Brix to 9.23, 9.65, 10.07, 10.33, and 10.47 °Brix at 0.05, 3, 6, 10, and 15% CO_2, respectively. These results also showed that the reduction in SSC was affected by the composition of the atmosphere ($p \leq 0.05$). The higher the CO_2 content (and the lower the O_2 content), the lesser the depletion.

The effect on SSC could be explained in terms of changes in berry metabolism. The increase in CO_2 content could reduce the respiration rate of these fruits and, therefore, the carbohydrate consumed to produce energy-rich ATP molecules. No studies on the effect of controlled atmosphere storage on the SSC of *Fragaria vesca* have been reported, though previous studies on cultivated strawberry varieties (*Fragaria ananassa*) did not report changes in the SSC associated with CO_2 partial pressure (*3*, *4*, *13*).

Titrable Acidity and pH. The titrable acidity values expressed as mass of citric acid per 100 mL of juice obtained for the different treatments and times of storage are presented in **Table 1**. As can be seen, values declined during storage from 0.996 to 0.590−0.751 mg/100 mL strawberry juice ($p \leq 0.05$). This acidity reduction during storage at low temperature was in agreement with the results showed for cultivated strawberries (*8*, *14*).

Differences between treatments were significant ($p \leq 0.05$) after three weeks of storage. The higher the CO_2 content (lower O_2) the higher the decrease in acidity. Thus, the reduction in

titrable acidity was about 0.25 mg/100 mL in air but over 0.4 mg/100 mL in a 15% CO_2-containing atmosphere. Gil et al. (*13*) reported a similar effect of controlled atmosphere on cultivated strawberries stored for 10 days at 5 °C. The acidity values after three weeks of storage depend linearly on CA compositions ($p \leq 0.001$, titrable acidity $= -0.0118[\%\ CO_2] + 0.7545$; $R^2 = 0.9562$).

As would be expected from the above-mentioned acidity values, wild strawberry pH values presented a slight increase (**Table 1**). Wild strawberries stored in air showed the lowest increase, only 0.03 pH units, while fruits stored in the 15% CO_2 atmosphere presented a pH increase of 0.3 units after three weeks of storage. These results were in agreement with those reported for cultivated strawberries (*4*, *14*−*16*). Although Brown proposed that the solubilization of CO_2 could produce a pH reduction (*17*), other authors have suggested that the effect of high CO_2 concentrations could affect organic acid metabolism and consequently the pH (*18*), an explanation that is in agreement with our results.

Attending to the titrable acidity and the soluble solid content results observed in this study, wild strawberries can be stored for three weeks at 3 °C, with acceptable quality, in any of the controlled atmosphere compositions tested (*19*).

Volatile Compounds. In this study, the evolution of the concentration of 17 organic volatile compounds present in strawberry aroma, comprising three fermentative metabolites (acetaldehyde, ethyl acetate, and ethanol) and 14 typical strawberry flavor compounds, was monitored during storage in the different controlled atmosphere conditions tested.

According to the literature, during storage, cultivated strawberries develop off-flavors which have been associated with the accumulation of acetaldehyde, ethanol, and ethyl acetate formed through anaerobic respiration pathways (*4*, *5*, *20*). **Figure 1** shows the initial concentration of the three metabolites in wild strawberries and their evolution during storage in the different controlled atmospheres. Ethanol and ethyl acetate presented the largest increases in concentration during storage (42- and 12-fold, respectively, at the atmosphere with the highest CO_2 content) and are probably the compounds responsible for off-flavors. In comparison, the contribution of acetaldehyde to off-flavors appears to be less significant (except in air).

Figure 1 also shows that the evolution of the three fermentative metabolites is affected by CA composition. Thus, the concentrations of ethanol and ethyl acetate increased during storage, with higher increments as the CO_2 partial pressure increased. This behavior was in agreement with most reports in the literature on cultivated strawberries and can be explained as a result of the anaerobic respiration induced by this gas. The contrary occurred for acetaldehyde, where the rise in concentration increased as the CO_2 partial pressure decreased, giving a final value in air three times higher than the values for the other CA conditions. This difference in the behavior of the three metabolites has not been reported for cultivated strawberries. Indeed, Larsen and Watkins (*7*) regarded acetaldehyde as one of the most prevalent products of anaerobic respiration. However, Smagula and Bramlage (*21*) reported that the accumulation of acetaldehyde that occurs in air-stored wild strawberries may be a result of the physiological breakdown caused by overripeness. Therefore, the lower acetaldehyde accumulation in CO_2-containing atmospheres may be an indication of improvement in prolonging wild strawberry shelf life. It is difficult to discover the reason for this unusual behavior. One hypothesis could be that the CA compositions may alter the activity of the different enzymes involved, that is, the presence of a high concentration of CO_2 and/or a low concentration of O_2 might decrease the activity of pyruvate decarboxylase in wild strawberries.

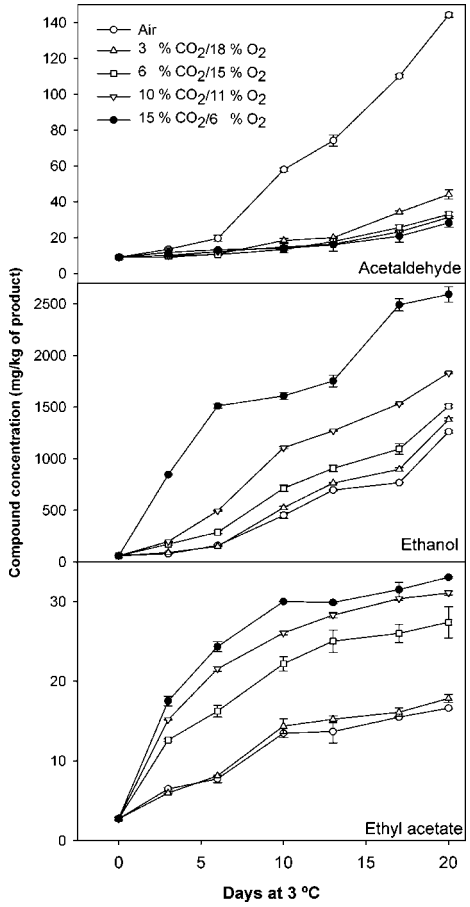

Figure 1. Changes in acetaldehyde, ethanol, and ethyl acetate concentrations (mg/kg strawberry) in wild strawberries over 20 days at 3 °C in five controlled atmosphere compositions.

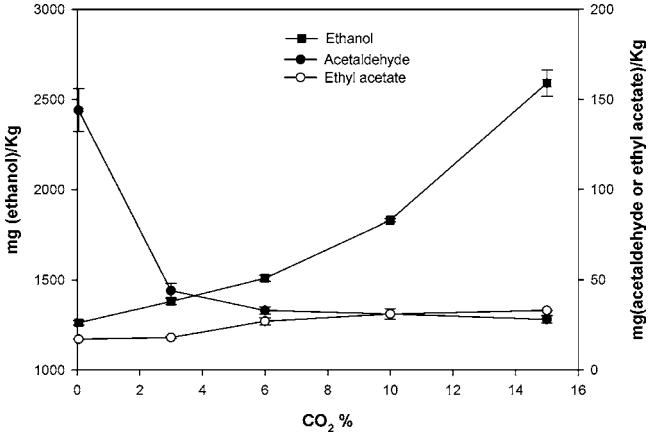

Figure 2. Concentration (mg volatile/kg fruit) of acethaldehyde, ethanol, and ethyl acetate in wild strawberries stored for three weeks in different concentrations of CO_2 at 3 °C.

Figure 2 shows the concentrations of the three compounds after three weeks of storage. At low CO_2 content (high O_2 percent) an excessive acetaldehyde concentration may spoil the fruits, and at high CO_2 partial pressures the concentration of ethanol increased exponentially. Therefore, wild strawberries appeared to be adequately stored in intermediate controlled atmosphere compositions with CO_2 concentrations between 3 and 10% (O_2 content between 18 and 11%).

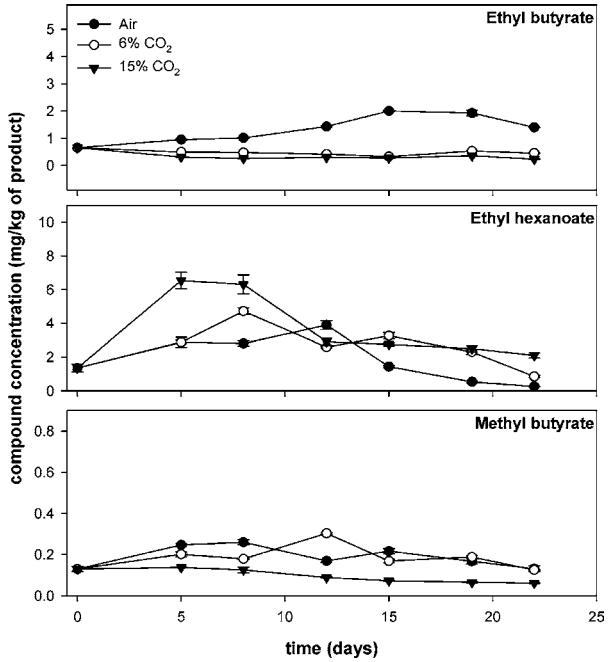

Figure 3. Evolution of ester concentrations in wild strawberries stored in three controlled atmosphere compositions.

Wild strawberry fruit aroma may be altered not only by the accumulation of fermentative metabolites but also by changes in the biosynthesis pathways of other volatile compounds, as happens in cultivated strawberries (*8*, *22*). Thus, selected volatile compounds of wild strawberries were analyzed over three weeks of CA storage at 3 °C. **Figures 3**−**6** present the results obtained for esters, acids, alcohols, and aldehydes/ketones, respectively. In these figures, day 0 values correspond to the concentrations at harvest of the different compounds in wild strawberry fruits. All the values lie within the same range of values as previously reported by Pyysalo et al. (*23*).

In contrast with the general increase in the concentration of fermentative metabolites, the concentration of volatile compounds does not present such a clear trend. Thus, **Figure 3** shows that ethyl and methyl butyrate concentrations presented a decrease during storage, with the exception of the ethyl ester in air, which increased. The concentration of these two esters also decreased as the CO_2 level rose. Ethyl hexanoate presented a considerable increase during the first days and a decrease after the first week of storage. The concentration of this compound at the end of the storage period increased with the CO_2 content. This effect on ethyl hexanoate has also been reported earlier in cultivated strawberries (*7*, *22*, *24*).

Two acids, acetic acid and hexanoic acid, were monitored; the results are presented in **Figure 4**. Acetic acid concentration increased in all cases, with the storage atmosphere composition having an unclear effect. Larsen and Watkins (*20*) observed an increase in acetic acid in cultivated strawberries exposed to air or high CO_2. The graph for hexanoic acid shows a different profile with a falling slope, less pronounced at higher CO_2 contents.

With respect to alcohols (**Figure 5**), *cis*-hexen-1-ol (hexenol), hexanol, citronellol, and benzyl alcohol present almost constant concentrations or slight depletion during storage, in agreement with Pelayo et al. (*25*). However, the hexenol content clearly increases during the last part of the storage period, a rise which is more pronounced for fruits stored in air. The contrary was found for benzyl alcohol and *B*-citronellol, as concentrations of these compounds decreased more when fruits were stored in air.

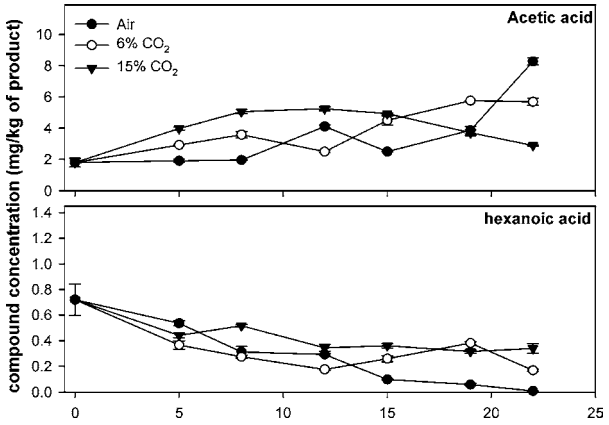

Figure 4. Evolution of carboxylic acid concentrations in wild strawberries stored in three controlled atmosphere compositions.

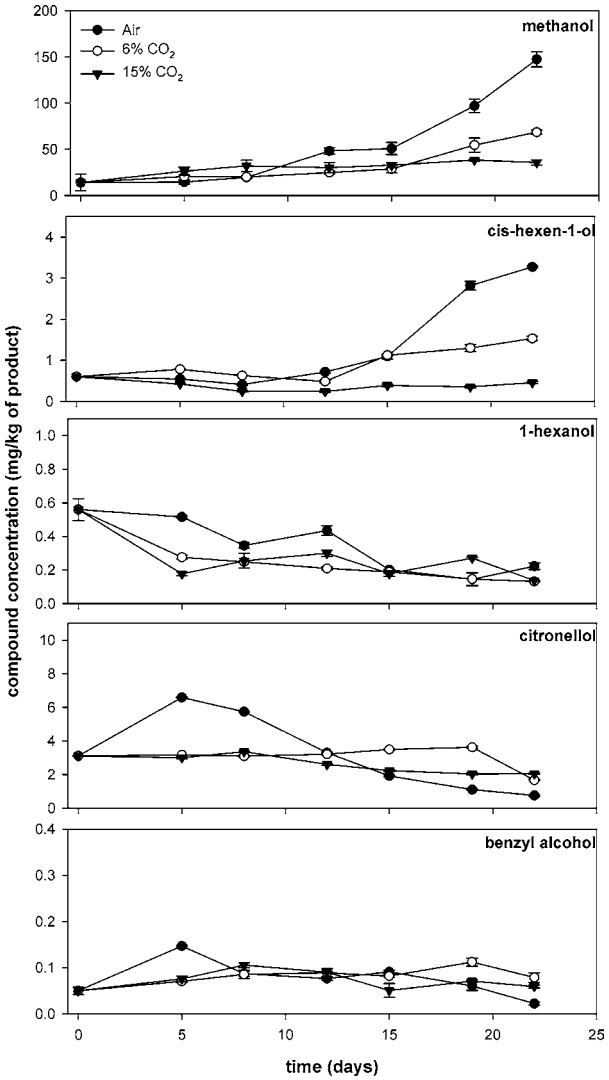

Figure 5. Evolution of alcohol concentrations in wild strawberries stored in three controlled atmosphere compositions.

Hexanal, 2-heptanone, 2-nonanone, and furanone were also monitored during storage. As seen in **Figure 6**, the general trend is a decrease in the concentrations of hexanal, 2-heptanone, and 2-nonanone, with the reduction being attenuated by the presence

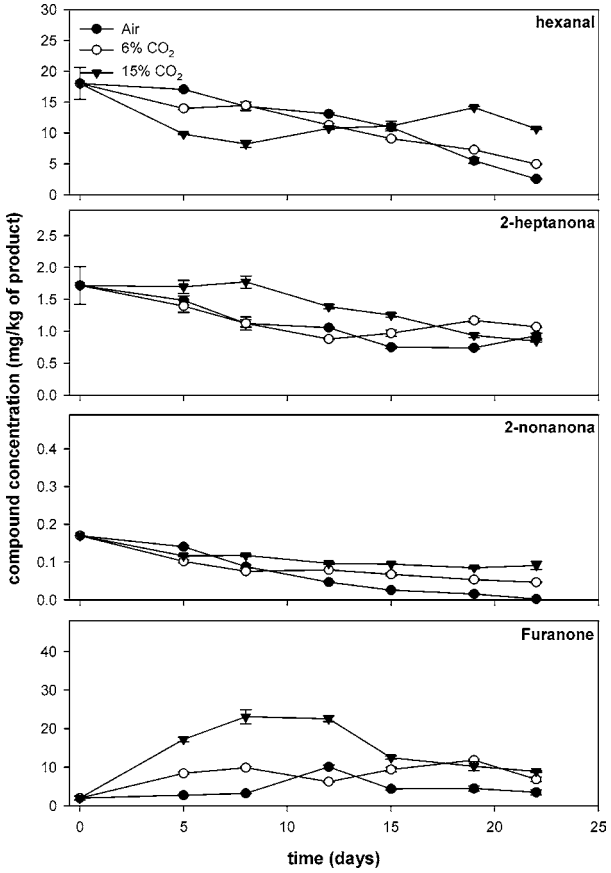

Figure 6. Evolution of aldehyde and ketone concentrations in wild strawberries stored in three controlled atmosphere compositions.

of CO_2. Nevertheless, furanone showed a significant increase in concentration, especially in the controlled atmospheres with the highest CO_2 content. Similar results were found in cultivated strawberries (*26*).

It is difficult to summarize the above comments, but the general effect of storage appears to be an increase in low-molecular-weight compounds (alcohols, aldehydes, esters) which could produce a faintly over-ripe flavor.

Visual Appearance. The fungistatic effects of high CO_2 levels on cultivated strawberries have been reported by several researchers (*3*, *27*). Exposure to CO_2 levels of between 5 and 20% have been reported to have some fungistatic effects on strawberry pathogens (*3*, *28*). Fungal infection on the surface tissue of the strawberries was visually estimated throughout the experiment, as this is the most evident deterioration process during postharvest storage (*29*, *30*).

In this study, the most obvious effect of CO_2-enriched atmospheres was a reduction in decay incidence; the effect of 15% CO_2 was the most pronounced. Wild strawberries stored in the lowest-CO_2 atmospheres (0.05, 3, and 6%) showed symptoms of *Botrytis cinerea* spoilage after 13, 15, and 17 days of storage, respectively, in agreement with El-Kazzaz et al. (*3*), who reported *Botrytis* development with physical damage and/or breakdown in storage at 3 and 6% CO_2. However, atmospheres with a high CO_2 content (10 and 15%) were successful in controlling the incidence of *Botrytis* throughout the entire test.

Sensory Evaluation. Sensorial evaluation was carried out after 10 days of storage. Fungal development impeded sensory tests after longer storage periods. The untrained taste panel did not find differences in quality attributes between treatments. Even when the extreme conditions were evaluated in triangular tests, non significant differences ($p \leq 0.05$) were detected in relation to taste and overall appearance. This result suggests that wild strawberry storage in high-CO_2 atmospheres does not cause significant changes in the taste or aspect of the fruits for at least 10 days.

Conclusion. The overall results indicate that wild strawberry shelf life can be effectively increased by exposing the fruits to a cold environment and an adequate atmosphere composition. The 10% CO_2/11% O_2 composition appears to prevent fungal growth, reduce the changes in soluble solids content and acidity, and maintain an acceptable aroma composition without triggering the concentration of fermentative metabolites.

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