INFLUENCE OF MODIFIED ATMOSPHERE PACKAGING STORAGE ON POSTHARVEST QUALITY AND AROMA COMPOUNDS OF STRAWBERRY FRUITS IN A SHORT DISTRIBUTION CHAIN

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ABSTRACT

Strawberry fruits (cv. Evie2) were stored at 18 ± 1 C for 2 days with a biobased and a polypropylene film in a passive and an active modified atmosphere packaging (MAP), and the packages were compared with a control (perfored) film. The effect of MAP on weight loss, color, flesh fruit firmness, soluble solids content, titratable acidity and aroma volatiles was monitored. Passive and active atmosphere with the biobased film resulted in low O₂ values. Fruits wrapped with the polypropylene film in the passive atmosphere were firmer than fruits stored in the other MAP condition and they exhibited the highest value of luminosity. Strawberries stored under active MAP condition with the biobased film showed the highest ester concentration (880.87 µg/kg) and ethyl was the dominant fraction. Considering the main qualitative traits and the aroma compounds measured, the perfored film was the only film that can be suggested to be used at 18 ± 1 C.

PRACTICAL APPLICATIONS

The modified atmosphere packaging (MAP) combined with high temperature could result in changes in quality and affect the main volatile compounds of strawberries along the storage. In this work, we evaluated two wrapping films (a polypropylene and a biobased film from starch corn) to store strawberries under different MAP conditions (active and passive) at high temperatures. Treatments able to maintain the most important qualitative traits and the aroma composition of fruits near to the harvest (0 day) could be considered when the cold conditions are not guaranteed in a distribution chain.

INTRODUCTION

A good way to maintain the supply chain for an innovative product is to keep production closer to the consumer (Salin 1998). The fundamental difference between the food supply chain and other supply chains is the continuous and significant changes in the quality of food products throughout the entire system until consumption. Short distribution chains and processing procedures can reduce the distance between producers and consumers, which is a good strategy to choose products linked to local food systems. Debates over alternative agrofood networks, food relocalization and the move to "quality" food production are thriving (Watts *et al.* 2005), with a focus on the transformative potential of embeddedness and local relationships of trust to stimulate changes in the agrofood sector (Winter 2003). Strawberries are universally recognized as having a basic chemical composition that accentuates their sweet taste, fruity aroma and healthy properties. The quality of fresh fruits as a function of their chemical compositions and organoleptic attributes is an important area of study. Ford *et al.* (1997) demonstrated that the quality of strawberries is the most important factor influencing retail sales, and they indicated that simple screening factors for compounds influencing taste

and aroma can be important for consumer acceptance. Kader (2008) reported that the longer the time between harvest and eating, the greater the loss of the characteristic aroma and the greater the development of off-flavors. Strawberries are very perishable and vulnerable to tissue damage during harvest and postharvest handling, as well as variations in storage conditions. Using controlled/modified atmospheres (Church 1994; Holcroft and Kader 1999) and controlling the relative humidity and rapid transport are important factors for improving the production of fresh fruits. To increase the consumption of fresh and healthy fruits, such as strawberries, it is necessary to control changes in volatiles and flavors that occur during marketing (Forney 2001; Kader 2008). Temperature control is essential for maintaining the flavor and quality of fresh produce (Tietel et al. 2012). Despite this, most innovations in traditional food products mainly pertain to the packaging (Gellynck et al. 2008). Modified atmosphere packaging (MAP) may be used to maintain the favorable environment within a sealed package until the product is sold, and it can be a supplement to proper temperature maintenance in the effort to delay ripening. MAP can be considered a self-contained form of controlled atmosphere designed to maintain the internal gas composition of the packaging during transportation and storage. A modified atmosphere extends the shelf life of the fruits, whereas the sealed container protects them from exposure to diseases and other environmental contaminants. The optimal gas composition of the MAP test for strawberries was 2.5% O2 and 16% CO2 (Sandhya 2010). Off-flavors can result from high CO₂ treatment, depending on the cultivar, temperature, exposure time and film permeability (Forney 2001; Peano et al. 2013). Different factors can influence the sorption of aroma compounds by the packaging: the type of packaging material, the nature of aroma compounds, the composition of the food matrices and the external environment (Ducret et al. 2001; Van Willige et al. 2003). Films slow down the movement of volatile aroma compounds, but the solubility coefficients and diffusivity of these compounds vary for different polymers (Charara et al. 1992). For example, low-density polyethylene films, which have been extensively studied, absorb a wide

variety of flavor compounds, such as aldehydes, methyl ketones, methyl esters and sulfur compounds (Arora *et al.* 1991; Letinski and Halek 1992). Some studies have compared the effects of biodegradable laminates and films on the quality of fresh produce (Makino and Hirata 1997; Del Nobile *et al.* 2006). However, until now, there were very few reports on the effectiveness of biodegradable film packaging on microbial and physicochemical quality during the storage of vegetables (Koide and Shi 2007). The aim of this work was to investigate the effect of MAP technology on the quality and volatile traits of cv. Evie 2 strawberries stored in a short distribution chain at the same temperature $(18 \pm 1C)$ used by retailers.

MATERIALS AND METHODS

Plant Material

"Evie2" strawberries were obtained from the commercial orchard Ortofruititalia soc.coop. S.R.L., Saluzzo (CN), Italy. The fruits were picked by hand at the end of August at the red ripe stage of maturity. They were graded for uniformity of color, and the damaged fruits were removed. They were manually picked into polyethylene terephthalate (PET) baskets and immediately transferred to the laboratory. The different storage treatments were started approximately 3 h after harvest.

Packaging Materials and Postharvest Storage

For the experiments, strawberry fruits were placed in PET baskets (0.250 kg per basket) and wrapped with three different single layer films under different gas compositions. For the flowpack equipment, an electronic horizontal wrapping machine (Taurus 800, Delphin, Malo, VI, Italy), including a take-up reel with translational jaw movement, was used. The gas was added using a PBI Dansensor (Segrate, MI, Italy) gas mixer that allowed for different gas mixtures (O_2 , CO_2 and N_2) to be used for each packed basket. Table 1

TABLE 1. DIFFERENT PACKAGES, FILM PERMEABILITY PROPERTIES AND THE RELATIVE ATMOSPHERE MODIFICATION USED IN THIS STUDY

Treatment	Modified atmosphere	Initial gas composition	Film packaging (25 μm)	O_2TR (ASTM F2622-08) at 23C and 50% RH	CO ₂ TR (ASTM F2476-05) at 23C and 50% RH
A	Passive	20.8% O ₂ + 0.2% CO ₂ + 78%N ₂	Biobased (from starch corn)	3,000	44,113
В	Active	10% O ₂ + 10% CO ₂ + 80% N ₂	Biobased (from starch corn)	3,000	44,113
С	Passive	20.8% O ₂ + 0.2% CO ₂ + 78% N ₂	Polypropylene (PP)	1,456	4,616
D	Active	10% O ₂ + 10% CO ₂ + 80% N ₂	Polypropylene (PP)	1,456	4,616
E	Control	20.8% $O_2 + 0.2\% CO_2 + 78\% N_2$	*Perfored (PP with 6-mm holes)	1,456	4,616

* The drilling of the polypropylene film does not influence the composition of the internal atmosphere of the packages. RH, relative humidity.

shows the films, the treatments (different packages), the permeability property and the relative modification of the atmosphere inside each basket.

The water vapor barrier of the biobased films, which are noncommercial films from starch corn (A and B treatments), was provided by the supplier (147 cm³/m² for 24 h), whereas the polypropylene film (C and D treatments) is classified as a high barrier material according to Van Tuil *et al.* (2000).

To simulate a short distribution chain that was similar to retailer conditions, the baskets were placed in a storage room at 18 ± 1 C for 2 days.

Sampling Method for Analyses

Three lots were used to determine the quality properties at harvest (0 days). The gas analysis and quality control tests were performed after 1 and 2 days of storage, and three randomly selected baskets (0.750 kg of strawberry fruits) were used for each treatment.

Headspace Gas Composition

The headspace gas composition analysis was performed on the entire basket. The changes in gas composition were measured daily over the course of the trial using a CO_2 and O_2 analyser (CheckPoint II, PBI Dansensor). To avoid modifications in the headspace gas composition due to gas sampling, the same air volume was maintained in the packages during the trial period as the analyzer introduced the same quantity of air that it removed for the analyses. To prevent gas leakage during the measurement, an adhesive septum (Septum white 150 mm, PBI Dansensor) was placed on the film's surface. The calibration was carried out using air (Aday and Caner 2011). The results are expressed as the average kilopascal of the three replicates.

Weight Loss and Fruit Color

The weight (water) loss of each package was measured using an electronic balance (SE622, VWR Science Education, MI, Italy) with an accuracy of 10⁻². The weight of each tray was recorded at harvest and at the end of each storage period. The weight loss is reported as the percentage of the initial fruit weight per tray. The results are expressed as an average of three replicates.

The color was measured on the first 10 healthy, nonmoldy fruits from each basket (three baskets were randomly chosen for each packaging). The mean of the 30 fruit measurements was used for data analysis. Color was measured on the side of a slightly flattened whole fruit using a tristimulus color analyzer (chroma meter, model CR-400, Minolta, Langenhagen, Germany) equipped with a measuring head with an 8-mm-diameter measuring area. The analyzer was calibrated to a standard white reflective plate (L = 97.26, a = +0.13, b = +1.71) and used Commission Internationale de l'Eclairage (CIE) Illuminant C. CIELAB or $L^*a^*b^*$ space was used to describe the color. This color space is device-independent and is able to create consistent colors regardless of the device used to acquire the image. L^* is the luminance or lightness component, and ranges from 0 to 100, whereas a^* (green to red) and b^* (blue to yellow) are two chromatic components, with values varying from -120 to +120 (Yam and Papakadis 2004). These values were used to calculate the chroma $(C^* = [a^2 + b^2]^{1/2})$, which indicates the intensity or color saturation, and hue angle $(h^{\circ} =$ arctangent[b^*/a^*]), where $0^\circ = \text{red}$ to purple, $90^\circ = \text{vellow}$, 180° = bluish to green and 270° = blue (McGuire 1992).

Flesh Fruit Firmness (FFF), Total Soluble Solids (TSS) and Titratable Acidity (TA)

The FFF was measured using a hand-held penetrometer (Effegi, Turoni, Forlì, Italy) with a calibration scale in grams and a 5-mm-diameter plunger, in accordance with standard industry practice. The head was pushed into the strawberry flesh to the depth of the head (5 mm). Two measurements each for 30 fruits were made on the opposite sides of the central zone and then averaged to give a mean value for the fruit. The measurements are reported in kg/cm².

TSS was measured using a digital refractometer (model PR-101, Atago, Tokyo, Japan) calibrated at +20C to 0% with distilled water. The prism surface and the light plate were washed and dried with a clean soft tissue paper between each reading. Two readings each for 30 fruits were taken and averaged. The values are expressed as °Brix at 20C.

The TA was measured using an automatic titrator (Titrino 702, Metrohm, Herisau, Switzerland). The TA was determined potentiometrically using 0.1 N NaOH to an end point of pH 8.1 in 5 mL of juice diluted with 50 mL of distilled water. Three measurements were made for each package.

Fruit Volatile Components

Volatile components were analyzed by the automatic headspace solid-phase dynamic extraction (HS-SPDE) technique combined with gas chromatography–mass spectrometry (GC-MS) and a CTC Analytics Combi PAL autosampler. The samples (three trays of 0.250 kg for each treatment) were blended to a fine puree. Then, 3 g of puree was placed into a 20-mL headspace glass vial (Brown-Chromatography Supplies, Wertheim, Germany) with 5 g of sodium chloride to increase the recovery of the extraction.

After equilibration at 35C for 15 min, the volatile components were extracted with an SPDE fiber (SPNdl-01/AC-50-56, PDMS + 10% active charcoal, 50 μ m, 57 mm) exposed to the headspace for 30 min. The fiber was then introduced into the injector of the GC for desorption at 250C for 3 min in the splitless mode.

Analysis of the volatile compounds was performed using an Agilent 6890 (Cernusco sul Naviglio, MI, Italy) GC equipped with a Restek Rxi-1MS column (30 m \times 0.25 mm i.d. \times 0.25-µm-thick film) and a mass spectrometer detector (Agilent 5973, Cernusco sul Naviglio, MI, Italy). The initial oven temperature was held at 0C for 2 min, increased by 5C/min to 100C and then raised by 4C/min to 230C, where it was held for 10 min.

Mass spectra were scanned from m/z 35 to 300, and volatile compounds were identified by comparing the mass spectra data with the Wiley library and retention times. The system was set up through injection of a standard mixture solution (terpenes/terpenoids, esters, aldehydes and hydrocarbons) in methanol at 0.01 µg/µL for each component: hexanal, isoamyl acetate, camphene, beta-pinene, limonene, gamma-terpinene, delta-3-carene, dodecane, menthol, carvone, alpha-copaene, pentadecane, hexadecane and heptadecane (Sigma-Aldrich, MI, Italy). The concentrations of the compounds were increased and the system linearity was verified under operational conditions, and good correlation coefficients ($r \ge 0.85$) were found.

The repeatability of the method (Peano *et al.* 2013) was investigated by performing three different injections from each 0.250-kg tray. A semiquantitative analysis was performed with an external standard calibration, with a focus on checking the retention time of each single component identified by the mass spectra.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) with a Tukey's test to determine the significance of difference of the means between the groups using the IBM-SPSS.20 software (2013). The differences were considered significant when P < 0.05.

RESULTS AND DISCUSSION

Headspace Composition

 O_2 and CO_2 levels (kPa) detected in the sample package headspace during storage are reported in Table 2. The initial gas composition changed rapidly for all treatments, and all films tested were able to create and to maintain MAP conditions. The exchange area of the film packages (550 cm²) and the temperature were kept constant and, therefore, the

TABLE 2. O_2 AND CO_2 CONCENTRATIONS IN THE HEADSPACE OF PACKAGED CV. EVIE2 STRAWBERRIES STORED AT 18 ± 1C AT DIFFERENT STORAGE TIMES

Variable		Storage time	age time (days)		
(kPa)	Treatment	0	1	2	
O ₂	А	20.8 ± 0.0	5.2 ± 2.3	0.9 ± 0.0	
	В	10.0 ± 0.0	1.2 ± 0.3	0.2 ± 0.1	
	С	20.8 ± 0.0	14.8 ± 0.6	13.3 ± 0.5	
	D	10.0 ± 0.0	0.8 ± 0.2	_	
	E	20.8 ± 0.0	20.8 ± 0.0	20.8 ± 0.0	
CO ₂	А	0.2 ± 0.0	11.9 ± 1.5	20.6 ± 0.9	
	В	10.0 ± 0.0	21.8 ± 1.5	26.0 ± 0.9	
	С	0.2 ± 0.0	6.0 ± 0.6	8.4 ± 0.5	
	D	10.2 ± 0.0	20.0 ± 1.1	_	
	E	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	

internal atmosphere change inside the packages was influenced by respiration of the fruits and the permeability of the films to O₂ and CO₂ (Beaudry et al. 1992). As expected, a decrease in the headspace O₂ concentration, as well as an increase in the headspace CO₂ concentration, was observed during storage for all of the treatments. Differences were observed between the gas levels established within the packages wrapped with different films since few hours from packaging (1 day). The partial O2 pressure decreased in fruits wrapped with the biobased film both in the passive and in the active MAP (A and B treatments), reaching 0.9 and 0.2 kPa, respectively, after 2 days. In contrast, the CO₂ concentrations increased up to similar values of 20.6 and 26.0 kPa, respectively, in the passive and active MAP packages (A and B treatments). The cause of the onset of anaerobic conditions achieved could suggest that the respiration rate of the strawberries at $18 \pm 1C$ occurs at a faster rate than the increase in permeation of the package, thus resulting in undesirable changes in the fruit, including the development of "off" flavors (Argenta et al. 2002). In passive MAP with the polypropylene film (C treatment), a steadystate level of 9-10 kPa was reached after 2 days. This confirmed the hypothesis that, while steady-state O₂ and CO₂ levels are determined by film characteristics and the produced respiration rate, the amount of time needed to achieve these levels depends on the initial atmospheric composition (Rodov et al. 2007).

Weight Loss and Fruit Color

The thin skin of the strawberries makes them susceptible to rapid water loss, resulting in shriveling and deterioration. Robinson *et al.* (1975) reported that losses of 6% of the initial value of fresh weight of a soft fruit should be considered the limit for marketability. After 2 days of storage, all samples had lost less than 2% of their fresh weight during all the storage time. In particular, the polypropylene film (C

treatment) resulted in the fruits maintaining a good state of hydration (data not shown). While Nunes *et al.* (2002) found that CO_2 delayed fruit coloring more effectively at 10C than at 4C, little is known about the effects of higher storage temperatures on fruits. Surface fruit color is a commercial indicator used to determine the ripeness of a strawberry (Civello *et al.* 1997), and it is one of the most important qualities of strawberries that is influenced by many factors, such as the fruit maturity, genotype and the cultivar (Wang and Camp 2000).

Changes in the color of strawberries were determined by *L*, *C* and h° values (Table 3), and all of the color parameters were significantly affected by the treatments. Change in the color of cv. Evie2 strawberries was found to be caused with decreasing brightness (L) and increasing redness, as indicated by decreasing chroma (C) and hue angle (h°) , respectively. After only 1 day of storage, strawberries become darker and, according to the literature (Almenar et al. 2007; Caner et al. 2008), all treatments showed L values lower than those obtained at harvest (40.47). The decrease in the L value reflects the darkening of the fruits, which was probably due the accumulation of anthocyanins, and indicates that the ripening process occurred in the fruits. After 2 days, fruits stored with the perfored film (E treatment) lost luminosity (i.e., showed a decreases in L^*) more rapidly than the fruits stored in MAP, which showed the lowest L^* value (35.73). Strawberries stored in passive MAP with the polypropylene film (C treatment) showed the highest L^* value (39.61), which resulted from the high CO₂ value observed in the headspace composition (Table 3). During storage, the color of the strawberries became less vivid (lower chroma) than at the time of harvest (45.99), and this trend was more evident for the fruit packaged with the perfored film (E treatment), which showed the lowest C value (38.10) after 2 days. This may be explained by oxidative browning reactions, which occur on strawberry surfaces in the presence of O_2 (Nunes *et al.* 2005; Aday *et al.* 2011). The degree in h° values was directly related to the humidity during storage (Goncalves et al. 2007). At the end of storage (2 days), fruits maintained in MAP (A, B and C treatments) showed higher values than fruits stored in the perfored film (E treatment), but no statistically significant differences were observed between the treatments.

FFF, TSS and TA

Studies on atmospheric control (El-Kazzaz *et al.* 1983; Smith and Skog 1992; Larsen and Watkins 1995) revealed the effect of high CO_2 atmospheres in maintaining or increasing initial firmness of strawberries stored at low temperatures. In our study, the high storage temperature (18 ± 1C) influenced the change in firmness of the stored

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TABLE 3. CHANGES IN THE FIRMNESS OF FRESH FRUIT, TOTAL
SOLUBLE SOLIDS, TITRATABLE ACIDITY AND COLOR OF CV. EVIE2
STRAWBERRIES STORED AT 18 \pm 1C FOR DIFFERENT AMOUNTS
OF TIME

	Days					
Treatments	0	1	2			
FFF (kg/cm ²)						
А		$0.58^{ns} \pm 0.09$	$0.70^{b} \pm 0.09$			
В		$0.60^{ns} \pm 0.09$	$0.70^{b} \pm 0.1$			
С	0.91 ± 0.13	$0.62^{\text{ns}}\pm0.09$	$0.82^{a} \pm 0.17$			
D		$0.58^{\text{ns}} \pm 0.1$	_			
E		$0.60^{\text{ns}}\pm0.09$	$0.83^{a} \pm 0.14$			
TSS (°Brix)						
А		$7.12^{ab} \pm 1.14$	$6.61^{b} \pm 0.84$			
В		$6.96^{b} \pm 1.07$	$6.9^{ab} \pm 1.1$			
С	6.36 ± 0.83	$7.72^{a} \pm 1.48$	$7.41^{a} \pm 1.74$			
D		$7.27^{\text{ab}}\pm0.94$	_			
E		$7.61^{a} \pm 0.92$	$7.42^{a} \pm 0.92$			
TTA (meq/L)						
А		$6.52^{\text{ab}}\pm0.03$	$6.56^{a} \pm 0.09$			
В		$5.92^{\circ} \pm 0.06$	$5.60^{\rm b} \pm 0.01$			
С	6.90 ± 0.12	$6.40^{\text{ab}}\pm0.22$	$6.51^{a} \pm 0.06$			
D		$5.50^{\circ} \pm 0.11$	_			
E		$6.07^{bc} \pm 0.05$	$6.66^{a} \pm 0.15$			
L		20 12b - 2 12	27 200 L 2 15			
A		$38.13^{\circ} \pm 3.13$	$37.28^{\circ} \pm 3.15$			
В	40.74 ± 5.07	38.62° ± 3.29	$37.01^{\circ} \pm 2.9$			
	40.74 ± 5.07	27 06b ± 2 25	39.01° ± 2.9			
		$37.30^{\circ} \pm 2.23$	- 25 72(± 2 57			
C		59.20°⊥2.54	55.75°±2.57			
A		$44.47^{ab} \pm 4.22$	$43.50^{a} \pm 3.76$			
В		$45.36^{\circ} \pm 3.16$	$44.49^{a} \pm 2.62$			
С	45.99 ± 4.06	$45.68^{\circ} \pm 4.11$	$43.46^{a} \pm 3.04$			
D		45.78° ± 3.25	-			
E		$43.29^{b} \pm 3.64$	$38.10^{b} \pm 5.45$			
h°						
A		$36.27^{ab} \pm 4.61$	35.5 ^{ns} ± 4.14			
В		$36.12^{ab} \pm 4.14$	35.88 ^{ns} ± 4.84			
C	37.26 ± 6.24	37.19° ± 4.27	37.27™±5.66			
D		35.96 [™] ± 2.65	-			
E		34.13° ± 3.97	35.19 ^{ns} ± 4.14			

The means in a column followed by different letters are significantly different at $P \le 0.05$ according to Tukey's test. ns, not significant.

fruit (Table 3). In particular, all treatments showed a decrease from the value of 0.91 kg/cm² observed for fruits at the harvest time (0 day) during all of the storage times due maturation development (García *et al.* 1998). As early as 1 day after statistically significant differences between the values obtained for fruits at harvest and for fruits stored with MAP were observed, but no differences were observed among the wrapping films. At the end of the storage (2 days), the firmness values were higher than after 1 day. This was probably due to the weight loss as water losses could

		Day 2							
Esters		Packaging condition	Packaging conditions						
(μg/kg)	Day 0	A	В	С	D	E			
Methyl esters	39.22 ± 1.2	189.74 ± 10.7	201.62 ± 11.0	149.85 ± 9.0	-	99.35 ± 8.0			
Ethyl esters	10.34 ± 0.9	470.66 ± 30.9	511.81 ± 41.2	13.61 ± 0.5	_	12.89 ± 0.2			
Other esters	31.65 ± 0.8	76.78 ± 10.9	167.44 ± 10.9	45.30 ± 0.9	-	39.05 ± 0.9			
Total	81.20	737.17	880.87	208.76	-	151.28			

TABLE 4. METHYL AND ETHYL ESTER LEVELS (EXPRESSED AS μ g/kg) IN CV. EVIE2 STRAWBERRIES STORED AT 18 ± 1C AFTER 2 DAYS OF STORAGE

Biobased film (A), biobased film + gas (B), polypropylene film (C), polypropylene film + gas (D) and polypropylene perfored film (E).

affect the hardness measure, as suggested by Johnson and Dover (2005). The lowest value (0.70 kg/cm²) was obtained with both the passive and the active MAP with the biobased film (A and B treatments) due to the reduced O_2 gas levels inside the packages.

Sweetness and acidity are two important components of the flavor of strawberries (Kader 2008). The TSS of cv. Evie2 strawberries at harvest was of 6.36° Brix and, as was previously reported (Peano *et al.* 2014), all treatments showed increases in the values during the storage time. This was likely due to weight loss, which, although minimal, greatly affects the concentration of total sugars (Table 3). The TA of cv. Evie2 strawberries decreased with the storage time when compared with the fresh fruits' values (6.90 meq/L) due to water loss during the 2 days of storage at the high temperature (data not showed). Considering the MAP conditions, the B treatment showed the lowest TA content after each storage time (5.92 and 5.60 meq/L), which corresponded to the lowest O₂ concentrations in the packages according to Picón *et al.* (1993).

Fruit Volatile Components

The selected aroma substances for each aromatic class were quantified daily, but the most interesting findings appeared

after 2 days. The mean values and standard deviations (μg / kg) obtained from the GC analyses of triplicate extractions are reported for all aroma compounds in Tables 4-7. In the literature, the number of aromatic volatile compounds in strawberries is more than 360 (Forney 2001). In this study, we detected a total of 61 volatile compounds from four different classes in cv. Evie2 (Table 8). Esters (38 compounds) were the most represented, followed by terpenes (13 compounds), aldehydes (7 compounds) and furanones (3 compounds). Strawberries have the ability to use volatiles from the air and metabolize them to produce volatile metabolites. This ability can be used to react alcohols with an acid to produce esters, reduce aliphatic aldehydes or reduce carbon-carbon double bonds in the carbonyl portion of aldehydes and ketones (Hamilton-Kemp et al. 1996). Quantitatively, the level of total aroma compounds varied among the treatments as a function of the MAP conditions. According to Pelayo et al. (2003), the volatile compound's aroma distribution is directly influenced by the high CO₂ treatments due to the direct action of the fungistatic effect of CO₂ accumulation inside the packages, and after only 2 days of storage, important differences between treatments are observed. As shown in Fig. 1, strawberries stored in the packages wrapped with the biobased film (A and B treatments) showed a similar aromatic pattern because the same

TABLE 5. ALDEHYDE LEVELS (EXPRESSED AS μ g/kg) IN CV. EVIE2 STRAWBERRIES STOREDAT 18 ± 1C AFTER 2 DAYS OF STORAGE

		Day 2				
Aldehyde	Packaging conditions					
(µg/kg)	Day 0	A	В	С	D	E
Hexanal	10.61 ± 1.0	14.46 ± 1.0	18.48 ± 1.1	18.87 ± 1.0	_	26.53 ± 1.3
(E)-2-Hexenal	9.77 ± 0.7	15.77 ± 0.9	13.91 ± 1.0	20.40 ± 0.9	_	23.19 ± 0.9
Heptanal	0.14 ± 0.01	0.34 ± 0.03	0.18 ± 0.02	0.23 ± 0.02	_	0.21 ± 0.03
2,4-Hexadienal	0.67 ± 0.03	0.99 ± 0.2	1.09 ± 0.3	2.42 ± 0.4	_	3.18 ± 0.8
2-Heptenal	0.17 ± 0.02	0.43 ± 0.02	0.33 ± 0.04	0.47 ± 0.01	_	0.57 ± 0.05
Nonanal	0.82 ± 0.05	1.15 ± 0.4	0.57 ± 0.1	0.98 ± 0.08	_	0.45 ± 0.06
Decanal	0.82 ± 0.07	0.84 ± 0.4	0.43 ± 0.08	0.99 ± 0.1	_	0.41 ± 0.1
Total	22.99	33.96	34.98	44.37	-	54.55

Biobased film (A), biobased film + gas (B), polypropylene film (C), polypropylene film + gas (D), and polypropylene perfored film (E).

		Day 2				
Terpenes components		Packaging condit	ions			
(µg/kg)	Day 0	A	В	С	D	E
Myrcene	0.56 ± 0.04	0.98 ± 0.05	0.53 ± 0.08	1.30 ± 0.1	-	6.63 ± 0.8
α -Phellandrene	0.61 ± 0.03	0.34 ± 0.01	0.26 ± 0.01	0.48 ± 0.03	_	0.53 ± 0.03
<i>p</i> -Cymene	0.17 ± 0.02	0.18 ± 0.02	1.11 ± 0.07	0.30 ± 0.04	_	0.25 ± 0.02
Limonene	0.77 ± 0.05	0.53 ± 0.06	0.24 ± 0.01	0.82 ± 0.03	_	0.99 ± 0.09
Sabinene	0.06 ± 0.0	0.13 ± 0.01	0.46 ± 0.05	0.15 ± 0.01	_	0.18 ± 0.01
β-Ocimene	0.41 ± 0.03	0.71 ± 0.04	0.67 ± 0.04	1.51 ± 0.1	_	1.43 ± 0.2
Terpinene	0.08 ± 0.0	0.25 ± 0.01	0.46 ± 0.08	0.40 ± 0.02	_	0.29 ± 0.02
Linalool oxide	0.00 ± 0.0	0.15 ± 0.01	0.34 ± 0.03	0.26 ± 0.03	_	0.26 ± 0.02
Linalool	2.42 ± 0.2	3.20 ± 0.2	4.00 ± 0.9	5.90 ± 0.9	_	6.54 ± 0.4
β-Farnesene	0.00 ± 0.0	0.10 ± 0.01	0.34 ± 0.02	0.56 ± 0.02	_	0.67 ± 0.01
α -Farnesene	0.19 ± 0.01	0.32 ± 0.04	0.42 ± 0.02	0.76 ± 0.03	_	0.77 ± 0.05
β-Bisabolene	0.33 ± 0.04	0.36 ± 0.03	0.28 ± 0.01	0.52 ± 0.04	_	0.42 ± 0.02
Bisabolene	1.08 ± 0.1	0.00 ± 0.0	0.23 ± 0.1	1.98 ± 0.2	_	2.28 ± 0.3
Total	7.28	8.28	10.43	17.38	-	23.43

TABLE 6. TERPENE LEVELS (EXPRESSED AS μq/kq) IN CV. EVIE2 STRAWBERRIES STORED AT 18 ± 1C AFTER 2 DAYS OF STORAGE

Biobased film (A), biobased film + gas (B), polypropylene film (C), polypropylene film + gas (D), polypropylene perfored film (E).

headspace gas composition was achieved inside these packages (Table 2).

ESTERS

The composition of volatiles in strawberries is dominated by esters, which account for 25-90% of the compounds (Forney et al. 2000), and they help to elevate the aroma of the strawberries by 50-200% during storage (Forney et al. 1998). Figure 1 shows that they range from 62.3 to 94.0% of the total aroma compounds, and 76% of the esters identified in the aroma of strawberries were found in cv. Evie2. At the time of harvest, the total volatile ester content was 81.2 µg/kg, but after 2 days of storage, the level had increased, to a large extent, as a result of the metabolism in the fruit tissue, which is in agreement with previous studies (Ulrich et al. 2007; Harb et al. 2008; Peano et al. 2014). The high value for the esters found for our experimental conditions were probably due the AAT enzyme, whose activity was shown to increase in strawberries during high temperature of storage (Pérez et al. 1996).

The ethyl ester fraction is dominant than the methyl fraction for the A (470.66 μ g/kg) and B (511.81 μ g/kg) treatments due to both the low O₂ concentrations and the high CO₂ levels inside the packages. In fact, the internal gas atmospheres during storage achieved with these two treatments caused an increase in pyruvate decarboxylase and alcohol dehydrogenase (Chang *et al.* 1983; Ke *et al.* 1994), which influences the ethanol concentration and leads to the formation of ethyl components (Ke *et al.* 1994; Tadege *et al.* 1999). For the C and E treatments, the ethyl ester contents were in the same range as fresh fruits (10.34 μ g/kg), and the total amount increase observed was primarily due to the methyl ester groups.

ALDEHYDES

The β -oxidation of fatty acids is one of the three main pathways for the production of volatile compounds in fruits, and it contributes to the origin of aldehydes. They are responsible for green odor notes, and their concentrations are typically related to fruit ripening. Although they

TABLE 7. FURANOE LEVELS (EXPRESSED AS μ g/kg) IN CV. EVIE2 STRAWBERRIES STORED AT 18 \pm 1C AFTER TWO 2 OF STORAGI
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		Day 2				
		Packaging con	idition			
Furanoes components (µg/kg)	Day 0	A	В	С	D	E
2-Amylfuran	0.39 ± 0.02	0.37 ± 0.03	0.44 ± 0.01	0.10 ± 0.01	-	0.00 ± 0.0
5-Ethyldihydro-2(3H)-furanone	0.44 ± 0.05	0.13 ± 0.01	0.20 ± 0.01	0.35 ± 0.04	-	0.25 ± 0.01
2,5-Dimethyl-4-methoxy-3(2H)-furanone (mesifurane)	1.53 ± 0.2	2.28 ± 0.2	5.26 ± 0.2	5.64 ± 0.3	-	7.33 ± 0.3
Total	2.36	2.77	5.90	6.10	-	7.58

Biobased film (A), biobased film + gas (B), polypropylene film (C), polypropylene film + gas (D), and polypropylene perfored film (E).

TABLE 8.	MAJOR V	OLATILE	COMPO	DUNDS	IDENTIFIED	in cv.	EVIE2
STRAWBEF	RIES FOR	ALL STC	RAGE	CONDIT	IONS		

Esters	Aldehydes
Ethyl caproate	(E)-2-Hexenal
Methyl hexanoate	Hexanal
Ethyl butanoate	2,4-Hexadienal
Ethyl 2-methylbutanoate	Nonanal
Isopentyl acetate	Decanal
Esenil acetate	2-Heptenal
Butyl acetate	Heptanal
Ethyl 3-methylbutanoate	Furanones
Octyl acetate	2-Amylfuran
2-Acetate esenil	5-Ethyldihydro-2(3H)-furanone
Ethyl 2-butenoate	2,5-Dimethyl-4-methoxy-3(2H)-furanone
Octyl isobutyrate	Terpenoids
Methyl octanoate	Myrcene
Ethyl 2-hexenoate	α-Phellandrene
3-Esenil acetate	<i>p</i> -Cymene
Benzyl acetate	Limonene
4-methyl methyl pentanoic	Sabinene
acid ester	β-Ocimene
Hexyl acetate	Terpinene
Amyl acetate	Linalool oxide
Ethyl benzoate	Linalool
Ethyl 3-hexenoate	β-Farnesene
Decyl acetate	α-Farnesene
2-esenil butyrate	β-Bisabolene
Phenethyl acetate	Bisabolene
3-Methylbutyl butanoate	
Ethyl cinnamate	
Propyl hexanoate	
Ethyl hexoate	
3-Methylbutyl octanoate	
Octyl propanoate	
Methyl 5-hexenoate	
Bornyl acetate	
2-Methylpropyl hexanoate	
Nonyl acetate	
Mitenil acetate	
Ethyl 3-hydroxyhexanoate	
Ethyl heptanoate	
Methyl 2-hydroxybutanoato	

dominate the aroma profile of immature fruit, the total aldehyde content does not disappear completely, but the concentration increases as ripening progresses (Table 5), as was reported by Pérez *et al.* (1999) and Azodanlou *et al.* (2004). The levels changed during the storage time, and after 2 days, all of the treatments showed higher values when compared to the fresh fruits (22.99 μ g/kg), thus suggesting that the ripening of all fruits was independent of the film packaging and the MAP conditions. The lowest total amounts were found for fruits wrapped with the biobased film stored with both the passive and the active MAP (A and B treatments; 33.96 and 34.98 μ g/kg, respectively). This was probably due to the reduced oxidation of fatty acids caused



FIG. 1. MAJOR VOLATILE AROMA COMPOUND DISTRIBUTION (%) IN CV. EVIE2 STRAWBERRY STORED AT 18 \pm 1C AT DIFFERENT STORAGE TIMES

by the development of anoxic storage conditions (Harb *et al.* 2008; Peano *et al.* 2014). The highest aldehyde level (54.55 μ g/kg) was found in fruits stored with the perfored film (E treatment). As reported previously (Jetti *et al.* 2007; Peano *et al.* 2014), the hexanal and the (E)-2-hexenal are the major components in fresh fruits (10.61 and 9.77 μ g/kg, respectively). However, for all MAP conditions, their levels were higher than those of other aldehydes during storage.

TERPENES

In our study, terpenoids represented 5.7% of the total aroma components of the stored fruit and were mainly represented by monoterpenes (C10) and sesquiterpenes (C15) (Table 6). As previously reported (Peano *et al.* 2014), the major component of the terpene fraction is linalool, which is also responsible for the typical strawberry smell (Larsen and Poll 1992). Interestingly, it has also been shown to be a highly potent antimicrobial substance (Ayala-Zavala *et al.* 2009). As was observed in other species (El Hadi *et al.* 2013), the decrease in O_2 levels and the increase in CO_2 levels observed for strawberries stored with the biobased films (A and B treatment) resulted in a lower capacity to synthesize terpenes when compared to fruits wrapped with the perfored film (E treatment), which showed the highest total terpene compound (23.43 µg/kg).

FURANOES

Furances represent 2.4% of the total aroma components of stored Evie2 strawberries. These compounds are unstable (Roscher *et al.* 1997), and their concentration is influenced by different growth conditions (Hirvi and Honkanen 1982) and cultivars (Douillard and Guichard 1990). 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4methoxy-3(2H)-furanone (mesifurane) are considered two of the most important aroma contributors (Jetti et al. 2007). In our study, they were found to be the most important components in both fresh and stored fruits (Table 7). According to previous studies (Forney 2001; Peano et al. 2014), for all MAP conditions, the content of 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) increased when compared with the value observed at harvest time $(2.36 \,\mu\text{g})$ kg). According to Pérez et al. (1996), the increase in furaneol derivative, such as the mesifurane, could indicate that the enzymatic system responsible for these processes is still active at 18 ± 1 C. Duan and Barringer (2012) found a correlation between furan formation and browning in sliced carrots during air-drying. Similarly, the total content of furances in our study was associated with a good correlation to a darker color (lower L value) of the strawberries (data not showed).

CONCLUSION

Maintaining the quality of highly perishable fruits, such as strawberries, associated with the concept of local and short chain systems is a difficult subject. The physicochemical parameter changes showed that the ripening process of cv. Evie 2 strawberries stored with MAP occurs during storage simulating retailer conditions. A storage temperature of $18 \pm 1C$ affected the metabolic activity of the fruits and the atmosphere composition inside the packages, and directly impacted changes in flavor compound synthesis and catabolism.

Although more than 360 compounds have been identified in strawberry aroma (Zabetakis and Holden 1997), in the Evie2 cultivar, only 61 volatiles appeared to be the most important contributors to the aroma of the strawberry, with esters being the most important class of compounds. Methyl and ethyl components increased for all MAP storage treatments, and the ethyl fraction production, as has been documented for other fruits (Fidler and North 1971; Yahia *et al.* 1992; Bender *et al.* 2000), could limit the marketability of fruits.

Low O_2 conditions that exceed the range of tolerance were observed for fruits wrapped with the biobased films (A and B treatments). These conditions could induce anaerobic metabolism and the accumulation of compounds such as acetaldehyde and ethanol, whose abundance could result in amounts of atypical off-flavors (Larsen and Watkins 1995). The amount of which would be needed to improve this work and to support the results obtained.

Considering all the qualitative parameters and aroma compounds measured, the perfored film (E treatment) showed the best result for storing fruits at 18 ± 1 C. In

general, it can be concluded that MAP conditions cannot be considered for strawberry fruit storage at the retailer temperature conditions for a short time. The perfored film (E treatment) was the only film that can be used in a short distribution chain, suggesting the role of the packaging to preserve fruits in terms of safety, manipulation and physical damage.

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