# Postharvest life and flavor quality of three strawberry cultivars kept at 5 °C in air or air + 20 kPa $CO_2$

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## Abstract

The postharvest life and flavor quality of three strawberry (*Fragaria x ananassa* D.) cultivars (Aromas, Diamante and Selva) kept at 5 °C in air or air + 20 kPa $CO_2$ for up to 15 days were investigated. 'Diamante' and 'Selva' had better flavor quality than 'Aromas' strawberries, as indicated by levels of titratable acidity and total soluble solids, organic acids, sugars and some aroma compounds and by a consumer preference test. Flesh firmness was maintained in 'Aromas' and increased in 'Diamante' and 'Selva' strawberries during storage at 5 °C in both air and air + 20 kPa $CO_2$. Fruit color was not affected by $CO_2$ treatments. The postharvest life based on appearance was 7, 9 and 9 days for 'Aromas', 'Diamante' and 'Selva' fruits stored in air and it was extended by 2, 2 and 4 days, respectively, by the $CO_2$-enriched atmosphere. However, the level and proportion of flavor components (sugars, organic acids, aroma compounds) and fermentative metabolites, as well as the results of sensory evaluations, indicated that the flavor life was shorter than postharvest life based on appearance in 'Aromas' fruit stored in air (5 vs. 7 days) and in $CO_2$-stored 'Aromas' (7 vs. 9 days) and 'Selva' (11 vs. 13 days) fruit. 'Selva' and 'Diamante' strawberries retained their flavor quality during storage at 5 °C in air for 9 days and $CO_2$-stored 'Diamante' fruit for 11 days.
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## 1. Introduction

The postharvest life of fruit and vegetables has been traditionally defined in terms of visual appearance (freshness, color and absence of decay or physiological disorders) and texture (firmness, juiciness and crispness). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it ignores flavor and nutritional quality. Flavor plays an important role in consumer satisfaction and influences further consumption of fruits and foods in general. Therefore, flavor, taste and aroma together with nutritional value (vitamins, minerals, dietary fiber and phytonutrients) should be incorporated into the

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postharvest life concept. Alternatively, new concepts, such as 'flavor life' and 'nutritional life', could be defined and considered in parallel with the primary concept of postharvest life.

The typical aroma of strawberries comes from not just one or a few impact aroma compounds, but from numerous volatiles present at certain concentrations and in a particular balance among them. Thus, strawberry aroma is the result of the combined perception of many aromatic notes, such as caramel, jam, floral, fruity, buttery, sour and grassy (Perez et al., 1993; Schieberle and Hofmann, 1997; Gomes da Silva and Chaves das Neves, 1999). Although $\approx 360$ compounds have been identified in the aroma of strawberries (Zabetakis and Holden, 1997), only 15–25 volatiles (methyl and ethyl esters, furanones, $C_6$ aldehydes and other $C_6$ derivative compounds, diacetyl, acetic acid and other aliphatic acids, linalool, $\gamma$-dodecalactone, benzaldehyde and some sulfur compounds) appear to be the most important contributors to strawberry aroma (Schieberle and Hofmann, 1997; Sanz et al., 1997; Zabetakis and Holden, 1997). Most of the above aroma compounds have been found in both wild and cultivated strawberry genotypes (Pyysalo et al., 1979; Larsen et al., 1992; Larsen and Poll, 1995). Apparently, it is the concentration and not the class of aroma compounds that is responsible for the large variation in aroma quality among cultivars (Hancock, 1999).

Strawberries also produce fermentative metabolites including acetaldehyde, ethanol and ethyl acetate under aerobic conditions (Li and Kader, 1989; Ueda and Bai, 1993; Larsen and Watkins, 1995a). These volatiles can impact the flavor if they are present in higher amounts than their flavor threshold values.

Modified atmospheres (MA) and controlled atmospheres (CA), containing elevated $CO_2$ concentrations extend the postharvest life based on the appearance and textural characteristics of strawberries (Harvey, 1982; Kader, 1991; Smith and Skog, 1992), but their effect on the flavor preservation of this fruit is not clear. Changes in pH, titratable acidity (TA), total soluble solids (TSS), sugars and organic acids and fermentative metabolites under the influence of $CO_2$-enriched

atmospheres have been reported (Gil et al., 1997; Holcroft and Kader, 1999b; Sanz et al., 1999). Elevated $CO_2$ favors the accumulation of acetaldehyde, ethanol and ethyl acetate (Li and Kader, 1989; Ke et al., 1991; Ueda and Bai, 1993; Larsen and Watkins, 1995a). This response is cultivar-dependent with only four out of seven strawberry cultivars accumulating high concentrations of these volatiles in air+20 kPa $CO_2$ at 2 °C (Watkins et al., 1999). An increase in ethyl esters other than ethyl acetate, with a concomitant reduction in other esters, has also been observed in elevated $CO_2$ in 'Chandler' (Ke et al., 1994) and 'Pajaro' (Larsen and Watkins, 1995b) strawberries. No changes in (E)-2-hexenal but an increase in acetic acid in air and to a greater extent, in elevated $CO_2$, were reported by Larsen and Watkins (1995b). Perez et al. (1996) reported an increase in the concentration of furanones in MA containing 6–9 kPa $CO_2$ and 13–16 kPa $O_2$ after 4–9 days at 0 °C in 'Oso Grande' strawberries, while Larsen and Watkins (1995a) did not find a similar effect in 'Pajaro' strawberries stored in 20 kPa $CO_2$ at 0 °C for 12 days.

In the present work, we determined postharvest life based on appearance and on changes in flavor components and sensory characteristics (flavor life) of three strawberry cultivars during storage in air or air+20 kPa $CO_2$. Our objective was to evaluate the potential of elevated $CO_2$ to preserve flavor as long as it maintains the appearance and textural quality of strawberries.

## 2. Materials and methods

### 2.1. Fruit source and experimental setup

Strawberries (cultivars: Aromas, Diamante and Selva) grown in Watsonville, CA were harvested at commercial ripeness ( > 75% of the surface showing red color), transported to UC-Davis in an air-conditioned car on the same day and placed at 0 °C overnight. The next morning, strawberries were sorted to eliminate fruit with defects, including overripe or too small fruit. Twenty-five fruits were selected randomly and placed in a 3.6-l glass jar as one replicate. Three replicates were used per

treatment. The jars were placed at 5 °C and ventilated with a continuous flow of humidified air or air enriched with 20 kPa $CO_2$ ($\approx 16$ kPa $O_2$) at a rate of 150 ml min$^{-1}$ using flow boards and capillary tubing as flowmeters. The atmosphere composition was analyzed daily with an infrared gas analyzer (HORIBA 2000R; Horiba Instruments, Irvine, CA) and adjusted to the desired composition.

## 2.2. Physiological and quality parameters

Respiration rates of air-stored fruit and ethylene production of fruit kept in air or air+20 kPa $CO_2$ were measured using an infrared gas analyzer and a flame-ionization gas chromatograph, respectively. For quality attributes, fruit samples were analyzed initially and every 2 days for a 15-day storage period. Visual quality and marketability (fruits with no symptoms of infections, dehydration or senescence) were evaluated on 15 fruit of each replicate. Those fruit in good condition were evaluated for color and firmness as previously reported (Pelayo et al., 2002). The same fruit were cut in small pieces, wrapped in cheesecloth, squeezed with a hand press and the clear juice was used for analysis. Total soluble solids, pH and titratable acidity were quantified as described by Gil et al. (1997) and the total concentration of anthocyanins by the spectrophotometric method described by Wrolstad (1976).

## 2.3. Phenolic compounds, sugars, organic acids and furaneol

Five whole fruit randomly selected from each treatment and replicate were directly frozen in liquid nitrogen and stored at −30 °C for subsequent analysis. Frozen strawberries were thawed and homogenized in a blender. Total phenolic compounds were quantified in 10 g homogenate samples using the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965); concentrations were calculated by preparing a *p*-coumaric acid standard curve. From the same homogenate, 10 g were used for the analysis of sucrose, glucose, fructose and citric and malic acids and 15 g for the analysis of furanones

(thermo-unstable aroma compounds) by using the HPLC methods previously described (Pelayo et al., 2002). Compounds were identified by retention times and quantified on the basis of peak areas of the corresponding standards.

## 2.4. Fermentative metabolites and aroma volatiles

Samples of 5 ml juice were placed in crimp-seal 10 ml vials containing 2 g of NaCl, sealed and frozen at −30 °C until the analysis of fermentative metabolites (acetaldehyde, ethanol and ethyl acetate) was conducted by GC using the static headspace technique previously described (Pelayo et al., 2002) with a 30 °C sample incubating temperature. Concentrations were calculated by using standard aqueous solutions of every analyte and by preparing the corresponding standard curves under the same conditions as those used for the strawberry samples. For aroma volatiles, 3 ml of an EDTA alkaline solution was added to 30 ml of the remaining strawberry juice to obtain a pH of 6.5 and a final EDTA concentration of 50 mM. Then, juice samples were frozen in liquid nitrogen and stored at −30 °C until the analysis of aroma compounds was conducted by GC-MS using the headspace-solid phase microextraction technique (Pelayo et al., 2002). Identification of aroma compounds was initially accomplished by matching mass spectra with library values. Confirmation of the identity of the major volatiles was performed by injecting standard aqueous solutions of each compound directly into the GC-MS and also by trapping the volatiles from the headspace by the DVB-SPME fiber under the same conditions as those used for the strawberry juice samples. Quantification was carried out by comparing peak areas of analytes to that of 2,6-dimethyl-5-heptenal added at 280 nl l$^{-1}$ as internal standard to the strawberry samples (Ulrich et al., 1995).

## 2.5. Sensory evaluation

In a second experiment, conducted 3 weeks later with strawberries of the same cultivars harvested from the same growing area and subjected to the same handling and storage conditions, sensory

evaluations were carried out. In a preference test conducted 48 h after the fruit were harvested, whole fruit from the three cultivars were ranked by randomly-selected, university-affiliated consumers according to their preference and results were analyzed by converting ranks to scores (1 = least preferred, 3 = most preferred). After 7 days of storage at 5 °C, comparisons were made between air- and $CO_2$-stored fruit by a discrimination duo–trio test (Larmond, 1970). The purpose of this test was to determine if panelists perceived differences in sensory characteristics between air- and $CO_2$-stored fruit. Results were analyzed by computing the correct judgments and by comparing this number with the number of correct answers reported in statistical charts (Larmond, 1970) for a 1 and 5% level of significance. After 9 days of storage, a discrimination paired comparison test (Larmond, 1970)—a more powerful discrimination test than duo–trio—was applied. In this test, panelists were asked for differences in astringency, sweetness, firmness, aroma and sourness. The same data analysis procedure used in duo–trio test was applied. Finally, after 11 days of storage, the strawberry-like flavor was scored in a scaling test by using a 10-point scale (0 = no strawberry-like flavor and 10 = intense strawberry-like flavor). Freshly-harvested strawberries were provided as a reference. Sensory evaluations, other than preference tests, were conducted with panelists in a special facility fitting the standard requirements for a testing room: booths with partitions to eliminate panelist interaction and facilitate concentration, quiet, odor free and illuminated with a green light to mask the strawberry color. In all tests, samples were presented coded with random numbers in identical containers and the order of presentation to each judge was randomized. The number of judges was 114 in the preference test, 18 in both duo–trio and paired comparison tests and 12 in the scaling test.

## 2.6. Statistical analysis

SAS system version 7.0 (SAS Institute Inc., Cary, NC) was used to perform analysis of variance (ANOVA) and to obtain LSD (5%) values of each of the main effects.

## 3. Results and discussion

### 3.1. Postharvest life and physiological parameters

The postharvest life at 5 °C, defined as the maximum storage period with nearly 100% of marketable fruit, was 7, 9 and 9 days for 'Aromas', 'Diamante' and 'Selva', respectively, in air and it was extended by 2, 2 and 4 days, respectively, by the $CO_2$-enriched atmosphere. Respiration rates in air of 'Aromas' and 'Diamante' averaged (6 mg $kg^{-1}$ $h^{-1}$ of $CO_2$), being slightly lower than 'Selva' (9 mg $kg^{-1}$ $h^{-1}$ of $CO_2$) strawberries at the beginning of the storage period. Similar ranking was observed at the end of postharvest life (7, 8 and 14 mg $kg^{-1}$ $h^{-1}$ of $CO_2$ for 'Aromas', 'Diamante' and 'Selva', respectively). In contrast, large differences in ethylene production were found among cultivars stored in air (Fig. 1). The highest initial rate was exhibited by 'Aromas' (112 nl $kg^{-1}$ $h^{-1}$) followed by 'Selva' (76 nl $kg^{-1}$ $h^{-1}$) and 'Diamante' (18 nl $kg^{-1}$ $h^{-1}$). Ethylene evolution gradually increased in air-stored fruit, but it was almost completely inhibited by the 20 kPa $CO_2$-enriched atmosphere. Since neither respiration rate nor ethylene produc-
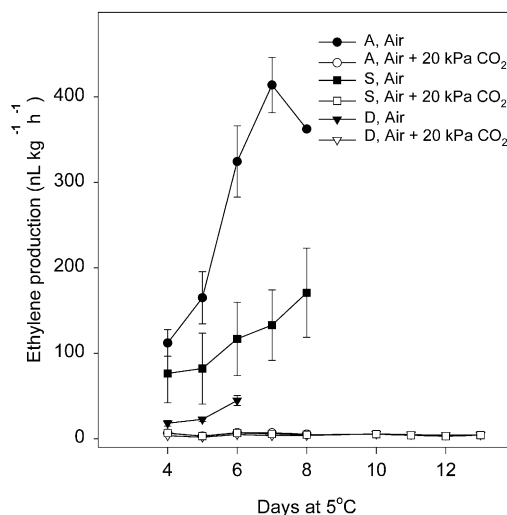


Fig. 1. Ethylene production (means ± S.D.) of three strawberry cultivars (A, Aromas; S, Selva; D, Diamante) stored at 5 °C in air or air + 20 kPa $CO_2$. Measurements were stopped when fruit became unmarketable.

tion were directly related to the postharvest life, we conclude that other factors play a role in determining the storage life of strawberries in air and its extension by $CO_2$. For example, the direct and indirect effect of low temperature on the growth of microorganisms and the effect of $CO_2$ on fruit firmness (Smith and Skog, 1992) and its fungistatic effect (Harvey, 1982) may also influence the storage life of these fruit.

## 3.2. Color and firmness

The three strawberry cultivars are characterized by differences in visual external color ('Aromas' = dark red, 'Diamante' = light red, 'Selva' = red). Hue angle (h) and the levels of anthocyanins (136, 67 and 106 mg l$^{-1}$, respectively) in the juice reflected these differences (Table 1). However, the distribution of these pigments in the fruit tissues of each cultivar is not uniform; the internal color of

'Aromas' and 'Diamante' strawberries is mostly white, whereas it is uniformly light red in 'Selva'.

Changes in color parameters during storage were cultivar dependent (Table 1). The color of 'Aromas' fruit became darker (lower L* values), less vivid (lower C* values) and tended to be more red (lower h values) with increased storage time. Similar trends were observed in 'Selva' strawberries during the postharvest life (up to 9 days of storage in air). In contrast, the color of 'Diamante' fruit tended to remain stable during storage and became darker (lower L* values) only after the end of postharvest life (11 days). High levels of $CO_2$ had no effect on these color parameters in 'Aromas' and 'Diamante' strawberries, but 'Selva' fruit stored under $CO_2$-enriched atmospheres tended to be brighter (higher L* value), more vivid (higher C* value) and less red (higher h value) in color than the corresponding air-stored fruit during most of the storage life (up to 11 days of storage). Anthocyanins levels in 'Aromas' and

Table 1
Quality attributes and flavor components of three strawberry cultivars stored at 5 °C in air or air+20 kPa $CO_2$

| Cultivar and days | Treatment | External color | | | pH | TA (%) | TSS (%) | Phenolics (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | | L | C | h | | | | |
| *Aromas* | | | | | | | | |
| 0 | – | 33.5 a[a] | 40.2 a | 26.0 a | 3.57 a | 0.72 a | 6.0 a | 1440 a |
| 7 | Air | 30.8 b | 37.4 b | 25.2 a | 3.60 a | 0.69 a | 5.8 a | 1640 a |
| | $CO_2$ | 33.2 a | 37.6 b | 24.9 a | 3.63 a | 0.69 a | 5.9 a | 1450 a |
| 9 | Air | 31.3 b | 38.2 b | 25.5 a | 3.58 a | 0.73 a | 5.8 a | 1480 a |
| | $CO_2$ | 31.1 b | 37.1 b | 24.2 a | 3.66 a | 0.66 a | 5.7 a | 1540 a |
| *Diamante* | | | | | | | | |
| 0 | – | 37.6 k | 44.0 k | 29.5 k | 3.59 k | 0.83 k | 6.8 k | 1360 k |
| 9 | Air | 38.0 k | 43.9 k | 29.9 k | 3.52 l | 0.83 k | 7.0 k | 1380 k |
| | $CO_2$ | 37.9 k | 45.8 k | 30.5 k | 3.58 l[b*] | 0.77 k | 6.9 k | 1400 k |
| 11 | Air | 36.4 k | 45.9 k | 29.8 k | 3.52 l | 0.82 k | 6.8 k | 1390 k |
| | $CO_2$ | 35.0 k | 42.5 k | 29.7 k | 3.57 l[b*] | 0.78 k | 7.0 k | 1310 k |
| *Selva* | | | | | | | | |
| 0 | – | 37.3 r | 44.0 r | 31.5 r | 3.59 r | 0.88 r | 8.2 r | 1360 r |
| 9 | Air | 36.9 r | 41.7 r | 29.6 r | 3.58 r | 0.89 r | 7.3 s | 1510 r |
| | $CO_2$ | 37.9 r | 46.3 r | 31.7 r | 3.62 r[b*] | 0.85 r[b*] | 7.4 s | 1630 r |
| 11 | Air | 36.6 r | 43.5 r | 30.8 r | 3.55 r | 0.91 r | 7.2 s | 1510 r |
| | $CO_2$ | 38.8 r | 44.0 r | 31.8 r | 3.62 r[b*] | 0.87 r[b*] | 7.3 s | 1470 r |
| 13 | Air | 37.5 r | 44.3 r | 31.4 r | 3.53 r | 0.92 r | 7.1 s | 1700 r |
| | $CO_2$ | 35.7 r | 42.8 r | 29.2 r | 3.65 r[b*] | 0.83 r[b*] | 7.3 s | 1360 r |

[a] Mean separation by LSD within columns (time effect) for each cultivar, $P \leq 0.05$.
[b] Mean separation by LSD between rows (air+20 kPa $CO_2$ effect), $P \leq 0.05$.

'Diamante' fruits were not affected by storage duration or atmosphere, but in 'Selva' the accumulation of anthocyanins observed in air-stored fruit was inhibited by $CO_2$ (Fig. 2). Similar results for 'Selva' were reported by Holcroft and Kader (1999a).

Similar initial flesh firmness (3.0 N) was measured in the three strawberry cultivars (Fig. 3). During storage, firmness changes were cultivar-dependent. Flesh firmness of 'Aromas' was not affected by storage time or $CO_2$, but that of 'Diamante' and 'Selva' increased over storage time and in response to high $CO_2$ levels. In the paired comparison test conducted after 9 days of storage, fruit stored in $CO_2$-enriched atmospheres were categorized as more firm than those stored in air, with significant differences at 5% level in 'Selva' (16 responses per 18 total). Several authors have reported increases in strawberry firmness by low temperature (Larsen and Watkins, 1995a; Watkins et al., 1999) and high $CO_2$ levels (Ueda and Bai, 1993; Larsen and Watkins, 1995b; Goto et al., 1996). Apparently, both the $CO_2$-firming effect and its magnitude are cultivar dependent (Smith and Skog, 1992; Watkins et al., 1999). The mechanism by which both low temperature and $CO_2$ affect strawberry firmness is not yet understood. An indirect effect of $CO_2$ on the apoplastic
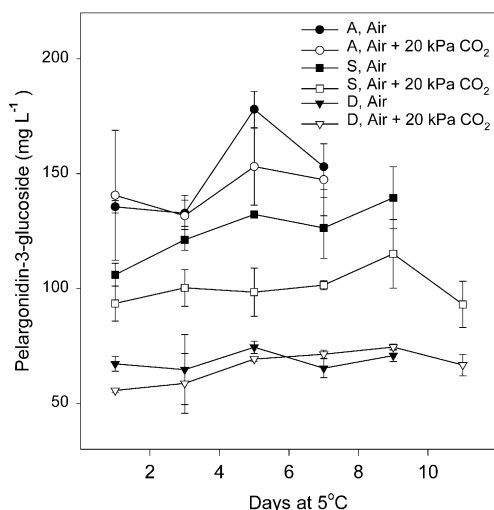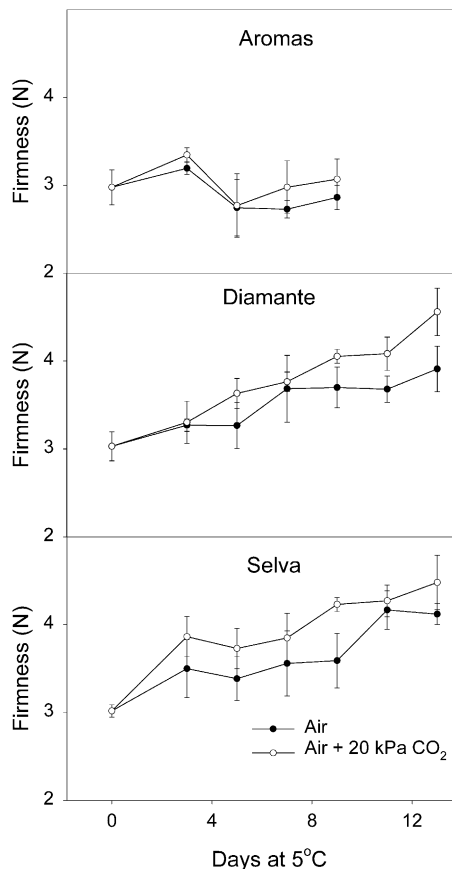


Fig. 3. Firmness (means±S.D.) of three strawberry cultivars stored at 5 °C in air or air+20 kPa $CO_2$.

pH with the subsequent precipitation of soluble pectins and the enhancement of cell-to-cell bonding (Harker et al., 2000) may be responsible for the observed firming effects.

### 3.3. Flavor components

#### 3.3.1. Organic acids, sugars and related parameters

The three cultivars had different TA, TSS, organic acid and sugar contents with 'Selva' and 'Diamante' fruit having the highest values (Tables 1 and 2). For an acceptable flavor, a maximum 0.8% TA and/or a minimum 7% TSS have been recommended (Mitcham et al., 1996). 'Aromas' strawberries fitted within the recommended TA value, 'Selva' fruit the recommended TSS, while



Fig. 2. Total anthocyanin content (means±S.D.) of three strawberry cultivars stored at 5 °C in air or air+20 kPa $CO_2$.

Table 2
Sugars and organic acids of three strawberry cultivars stored at 5 °C in air or air + 20 kPa $CO_2$

| Cultivar and days | Treatment | Sucrose (%) | Glucose (%) | Fructose (%) | Total sugars (%) | Citric acid (%) | Malic acid (%) | Total acids (%) |
|---|---|---|---|---|---|---|---|---|
| *Aromas* | | | | | | | | |
| 0 | – | 0.78 a[a] | 1.50 a | 1.84 a | 4.12 a | 0.55 a | 0.21 a | 0.77 a |
| 7 | Air | 0.34 b | 1.61 a | 2.12 a | 4.07 a | 0.66 a | 0.18 bc | 0.84 a |
| | $CO_2$ | 0.33 b | 1.37 a | 1.74 a | 3.45 b | 0.49 a | 0.12 bd[b*] | 0.61 a |
| 9 | Air | 0.28 b | 1.50 a | 1.99 a | 3.78 b | 0.64 a | 0.16 bc | 0.80 a |
| | $CO_2$ | 0.32 b | 1.44 a | 1.84 a | 3.59 b | 0.53 a | 0.12 bd[b*] | 0.65 a |
| *Diamante* | | | | | | | | |
| 0 | – | 1.48 k | 1.69 k | 2.05 k | 5.22 k | 0.66 k | 0.23 k | 0.89 k |
| 9 | Air | 0.70 ln | 1.73 k | 2.14 k | 4.56 k | 0.75 k | 0.13 lm | 0.88 k |
| | $CO_2$ | 1.00 lm[b*] | 1.95 k | 2.30 k | 5.28 k | 0.66 k | 0.13 lm | 0.79 l |
| 11 | Air | 0.50 ln | 1.74 k | 2.16 k | 4.41 l | 0.72 k | 0.16 lm | 0.88 k |
| | $CO_2$ | 0.76 lm[b*] | 1.60 k | 1.96 k | 4.31 l | 0.66 k | 0.10 ln | 0.75 l |
| *Selva* | | | | | | | | |
| 0 | – | 1.63 r | 1.55 r | 1.95 s | 5.13 r | 0.72 r | 0.28 r | 1.00 r |
| 9 | Air | 0.97 st | 1.74 r | 2.28 r | 4.99 r | 0.71 r | 0.26 r | 0.97 r |
| | $CO_2$ | 0.97 st | 1.88 r | 2.32 r | 5.17 r | 0.70 r | 0.20 st[b*] | 0.90 r |
| 11 | Air | 0.69 su | 1.68 r | 2.19 r | 4.56 s | 0.77 r | 0.21 st | 0.98 r |
| | $CO_2$ | 0.73 su | 1.65 r | 1.98 s[b*] | 4.36 s | 0.62 r | 0.17 st[b*] | 0.79 r[b*] |
| 13 | Air | 0.51 su | 1.63 r | 2.17 r | 4.31 s | 0.85 r | 0.22 st | 1.10 r |
| | $CO_2$ | 0.59 su | 1.49 r | 1.88 s[b*] | 3.96 s | 0.59 r | 0.13 su[b*] | 0.72 r[b*] |

[a] Mean separation by LSD within columns (time effect) for each cultivar, $P \leq 0.05$.
[b] Mean separation by LSD between rows (air + 20 kPa $CO_2$ effect), $P \leq 0.05$.

'Diamante' strawberries were close to both the recommended TA and TSS.

CO_2-stored 'Selva' strawberries were less acidic than when stored in air as indicated by pH, TA and levels of malic and total organic acids (Tables 1 and 2). Similar trends were observed in 'Aromas' and 'Diamante' fruit but only some juice acidity measurements were statistically different. Similar results were reported by Ke et al. (1991) and Holcroft and Kader (1999b) for 'Selva' strawberries, but panelists, in a paired comparison test conducted after 9 days of storage, did not detect any significant treatment effects for sourness for this cultivar. Probably, the quantitative differences in juice acidity were not large enough to be detected by sensory perception, especially in the presence of other flavor components. In the three cultivars, citric was the dominant organic acid (72–74% of total acids) and during storage, only malic showed a consistent decrease with storage time and high $CO_2$ levels. Holcroft and Kader (1999b) found that levels of citric acid increased in external but not in internal tissues of 'Selva' strawberries stored at 5 °C in air + 20 kPa $CO_2$, while malic acid decreased in both external and internal tissues.

TSS decreased during storage time only in 'Selva' fruit (Table 1), but the three cultivars had reduced total sugars with storage time. Sugars were not affected by atmosphere. No significant differences in sweetness between air- and CO_2-stored fruit of the three cultivars were detected. Sucrose (present at the beginning of storage at 19, 28 and 34% of total sugars in 'Aromas', 'Diamante' and 'Selva' fruit, respectively) was at least 50% hydrolyzed by the end of postharvest life with a significantly lower rate of sucrose hydrolysis only in 'Diamante' fruit stored in a CO_2-enriched atmosphere (Table 2). In contrast, glucose and fructose decreased only by 1–12% after 9, 11 and 13 days of storage in 'Aromas', 'Diamante' and 'Selva' fruit, respectively, with nonsignificant differences between air- and CO_2-stored strawberries, except at 11 and 13 days in 'Selva' fruits, where

concentrations of fructose were lower in $CO_2$- than in air-stored fruit. Thus, the observed reduction of total sugars during storage was mostly explained by the hydrolysis of sucrose and the utilization of the corresponding reducing sugars in the fruit respiration.

At the end of postharvest life, the only cultivar fitting both the proposed maximum TA (0.8%) and minimum TSS (7%) for an acceptable flavor was 'Diamante' stored in air$+CO_2$. 'Aromas' fruit fitted the proposed TA but had a lower TSS, and 'Selva' fruit fit the proposed TSS but had a higher TA in both air and air$+CO_2$, while 'Diamante' fruit stored in air had the proposed TSS and a TA close to the proposed value.

### 3.3.2. Phenolic compounds

The level of total phenolics ($1 \cdot 36$–$1 \cdot 44$ g kg$^{-1}$) was similar among the three cultivars (Table 1). No significant differences in the level of phenolics in relation to storage atmosphere were found in fruit of any cultivar. Also, no relationship was found between the phenolics content and the sensory perception of astringency. In the paired comparison test conducted after 9 days of storage, panelists found that the air-stored 'Diamante' fruit were significantly more astringent than their corresponding $CO_2$-stored fruit. Since tannins are the phenolics most directly related to astringency and those corresponding to the category of hydrolyzable tannins are dominant in strawberries (Foo and Porter, 1981), the direct quantification of these compounds may provide a better agreement with astringency.

### 3.3.3. Fermentative metabolites

In freshly harvested strawberries, the level of fermentative metabolites was low (44, 32 and 29 μl l$^{-1}$ in 'Aromas', 'Diamante' and 'Selva', respectively). Changes of these compounds during storage were dependent upon cultivar, storage time and atmosphere conditions. In 'Aromas', 'Diamante' and 'Selva' strawberries stored in air, the levels of fermentative metabolites did not change up to the end of postharvest life (Fig. 4). However, the concentration of fermentative metabolites increased in 'Aromas' and 'Selva' fruits kept in air$+CO_2$. In contrast, the level of these com-
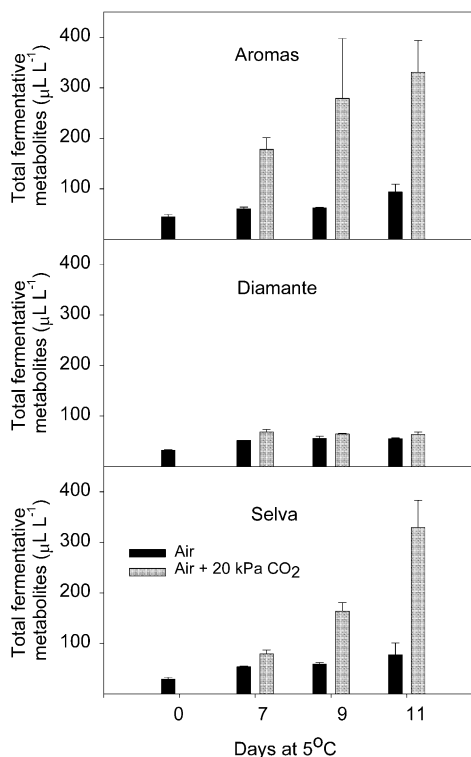


Fig. 4. Fermentative metabolites (means$\pm$S.D.) of three strawberry cultivars stored at 5 °C in air or air$+20$ kPa $CO_2$.

pounds remained stable in air$+CO_2$-stored 'Diamante' fruits. Watkins et al. (1999) reported a similar cultivar variation in the accumulation of fermentative metabolites in response to high $CO_2$ levels.

### 3.3.4. Aroma compounds

Cultivars differed quantitatively in aroma compounds, but produced the same major aroma compounds (esters and furanones) (Table 3). Among minor aroma compounds, acetic acid (sour aroma) was the only volatile not detected in 'Aromas' strawberries. The branched esters ethyl-2-methyl propanoate and methyl/ethyl-2-methyl butanoate, the aliphatic acid butanoic acid (sweaty aroma) and the ketone 2,3 butanedione or diacetyl (buttery aroma), considered important contributors to the strawberry flavor by Schieberle (1994) and Schieberle and Hofmann (1997), were not detected before storage of the

Table 3
Qualitative aroma composition of 'Aromas', 'Diamante' and 'Selva' strawberries before storage

| Methyl esters | $C_6$ Aldehydes |
|---|---|
| Methyl butyrate | Hexanal |
| Methyl hexanoate | (E) 2-hexenal |
| Ethyl Esters | $C_6$ Alcohols |
| Ethyl butyrate | 2 ethyl 1-hexanol |
| Ethyl hexanoate | (E) 2-hexenol |
| Other Esters | Aliphatic acids |
| Butyl acetate | Acetic acid[b] |
| Butyl butanoate | |
| Hexyl acetate | Aromatic aldehydes |
| (E) 2-hexenyl acetate | Benzaldehyde |
| 2-Methyl butyl acetate[a] | |
| Furanones | |
| Furaneol [2,5-dimethyl- 4-hydroxy-3(2H)-furanone] | |
| Furaneol glucoside | |

[a] Only detected in 'Aromas' and 'Diamante'.
[b] Only detected in 'Diamante' and 'Selva'.

cultivars. However, branched esters were found in $CO_2$-treated fruit probably related to stress conditions or senescence, as previously discussed for 'Camarosa' (Pelayo et al., 2002).

Quantitatively, large differences in the level of total aroma compounds (TAC) were found among the cultivars (Fig. 5). TAC was similar in 'Aromas' and 'Selva' (478 and 479 nl $l^{-1}$, respectively), but it reached 1011 nl $l^{-1}$ in 'Diamante' fruit. This high level of TAC in 'Diamante' was due to a larger amount of all groups of aroma compounds. Esters (most characterized by fruity aromatic notes) were the most dominant aroma compounds in the cultivars.

Concentrations of $C_6$ aldehydes and $C_6$ alcohols (grassy or herbaceous aromatic notes) were similar in 'Aromas' and 'Selva' (112 nl $l^{-1}$) strawberries, but lower than in 'Diamante' fruit (181 nl $l^{-1}$). The concentration of benzaldehyde varied greatly among the three cultivars: 12, 32 and 122 nl $l^{-1}$ (3, 3 and 26% of TAC), in 'Aromas', 'Diamante' and 'Selva', respectively. Acetic acid averaged only 12.5 nl $l^{-1}$ in 'Diamante' and 'Selva'. The lowest level of furanones (furaneol, characterized by a caramel-like or jam aromatic note and furaneol glucoside) was found in 'Aromas' (5.3 mg $kg^{-1}$)

followed by 'Selva' (7.4 mg $kg^{-1}$) and 'Diamante' (7.7 mg $kg^{-1}$) fruits.

Changes in aroma compounds during storage were also cultivar, storage condition and duration dependent. In 'Aromas', the level of TAC increased at the end of postharvest life in both air and $CO_2$ (Fig. 5). In contrast, TAC decreased in 'Diamante' fruit stored in air and to a higher extent those kept in air+$CO_2$ (Fig. 5). In 'Selva' TAC increased only in air-stored fruit. Major changes in TAC during storage could be explained by modifications in the levels of esters.

In air-stored fruit, the level of ethyl esters at the end of postharvest life (7 days) of 'Aromas' increased while the concentration of methyl and other esters remained essentially the same (Fig. 5). In 'Selva' only a slight increase in the concentration of ethyl esters and a larger increase in methyl and other esters was observed after 9 days of storage (Fig. 5). A low level of ethyl esters was observed in 'Diamante' strawberries after 9 days of storage. At the end of postharvest life in air, ethyl, methyl and 'other esters' were present in 'Aromas' strawberries, while only methyl and 'other esters' were found in 'Selva' and 'Diamante' fruit.

In $CO_2$-stored strawberries, the most notable change during storage was a large increase in the concentration of ethyl esters in 'Aromas' and 'Selva' fruit (Fig. 5). In contrast, the levels of methyl and other esters, which were maintained in 'Aromas' and increased in 'Selva' fruit stored in air, decreased in both cultivars under elevated $CO_2$. Ke et al. (1994) found that the level of ethyl butyrate increased while the level of three acetates decreased in 'Chandler' strawberries stored at 5 °C in 50 kPa $CO_2$. Levels of esters in $CO_2$-stored 'Diamante' fruit followed the same trend as in air (Fig. 5). Thus, at the end of postharvest life, ethyl esters dominated over methyl and other esters in 'Aromas' and 'Selva', but not in 'Diamante' strawberries in which the last two fractions were present in smaller amounts, but in similar proportions to those at the beginning of storage (Fig. 5).

An enhanced production of ethyl esters must require an enhanced fermentation process to supply higher amounts of the precursor ethanol. Fermentative metabolism can be enhanced in
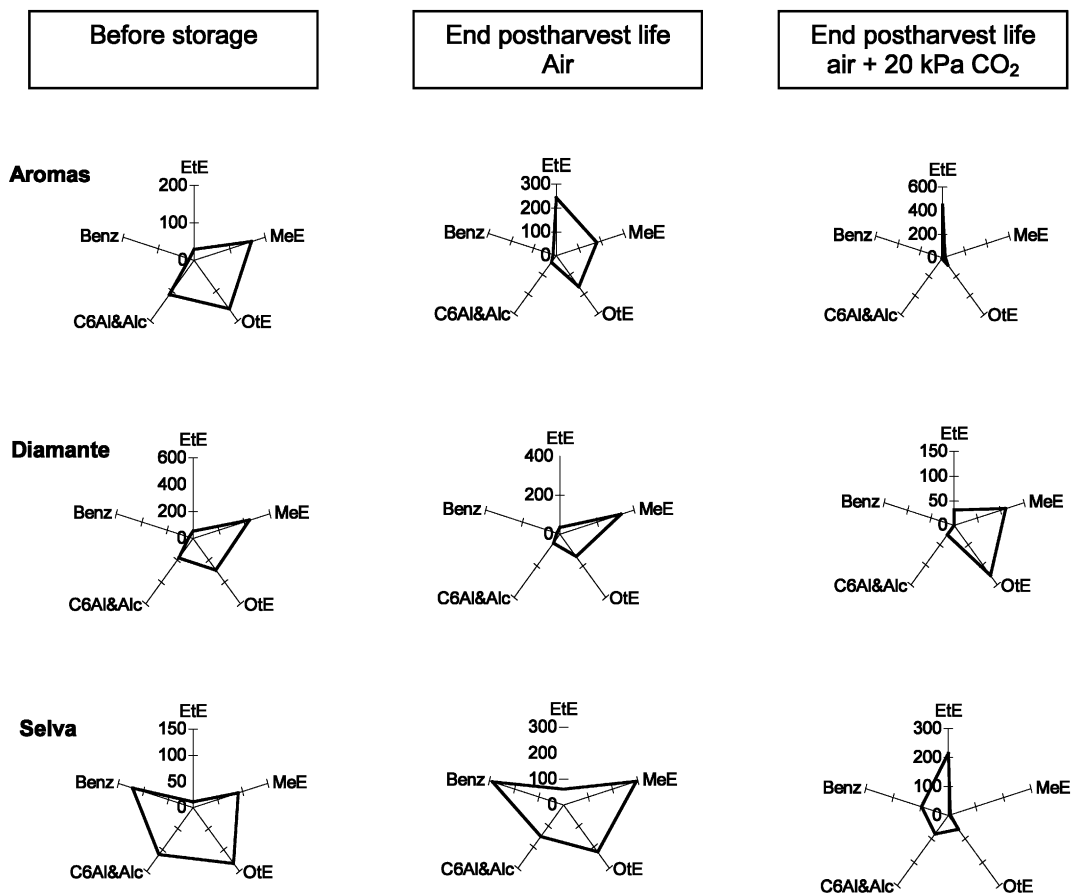
Fig. 5. Aroma profiles of three strawberry cultivars before storage and at the end of postharvest life at 5 °C in air or air+20 kPa $CO_2$ (EtE, ethyl esters; MeE, methyl esters; OtE, other esters; $C_6$Al&Alc, $C_6$ aldehydes and $C_6$ alcohols; Benz, benzaldehyde in nl l$^{-1}$).

fruits by several stress factors including environmental (chilling injury temperature, hypoxic conditions), biotic (microbial infections) and internal (ripening, senescence) factors (Purvis, 1997). Apparently, senescence was the factor increasing the synthesis of ethyl esters in 'Aromas' fruit after 7 days of storage in air, while $CO_2$ was the factor enhancing the production of ethyl esters in 'Aromas' and 'Selva' fruit. Under high rates of ethyl ester production, the synthesis of methyl and other esters is limited and deviations from the original aroma profiles are favored. The preferential synthesis of ethyl esters over other esters in the presence of high levels of ethanol is possible because the activity of the enzyme alcohol acyltransferase (AAT) apparently depends more on substrate availability than on substrate specificity (Aharoni et al., 2000). Thus, apparently the ability to maintain the original ester profile during storage depends on the ability of cultivars to maintain a low rate of fermentative metabolism. 'Aromas' did not maintain a low rate of this metabolic pathway in either air or $CO_2$, 'Selva' maintained a low rate of fermentation process in air but not in $CO_2$ and 'Diamante' was able to maintain a low fermentation process in both air and $CO_2$.

The biochemical basis of this cultivar variation in aroma has not yet been elucidated. Differences may be due to natural variation among cultivars in the activity of fermentative- and volatile biosynthesis-related enzymes and isoenzymes in response to senescence and stress conditions. In 'Chandler', a

cultivar which accumulates fermentative metabolites under $CO_2$, Ke et al. (1994) found an increase in the activities of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) and a decrease in the activity of AAT, the enzymes catalyzing the synthesis of acetaldehyde, ethanol and ethyl acetate, respectively, in response to an atmosphere of 21 kPa $O_2$+50 kPa $CO_2$. However, according to Fernández-Trujillo et al. (1999), the activity of PDC and ADH only increased in those cultivars of strawberries that are able to maintain a low level of fermentative metabolites when exposed to $CO_2$-enriched atmospheres.

The level of $C_6$ aldehydes and alcohols at the end of the postharvest life decreased in air-stored fruit and to a greater extent, in $CO_2$-stored 'Aromas' and 'Diamante' strawberries. In 'Selva' fruit, the concentration of these $C_6$ compounds was maintained in air, but decreased under elevated $CO_2$ (Fig. 5). The concentration of benzaldehyde, which was exceptionally high at 120 nl $l^{-1}$ (26% of TAC) in 'Selva' fruit at the beginning of the storage, ranged from 70 to 288 nl $l^{-1}$ (18–26% of TCA) in air and from 78 to 126 nl $l^{-1}$ (9–14% of TCA) in $CO_2$ after 7–11 days at 5 °C. In the other two cultivars, this aromatic aldehyde did not show clear trends during storage, but tended to decrease in the $CO_2$-enriched atmosphere. The level of furanones increased 2-fold at the end of postharvest life in 'Aromas' and 'Selva' strawberries (10 mg $kg^{-1}$) and 2.5-fold in 'Diamante' fruit (17 mg $kg^{-1}$) without significant differences between air and $CO_2$ stored fruit.

The described changes in aroma compounds during storage created new aroma profiles that might influence the flavor perception of these fruit. However, a discrimination duo–trio test conducted after 7 days of storage indicated that only air-stored 'Aromas' fruit were significantly different from their corresponding $CO_2$-stored fruit in terms of overall sensory perception at 1% level of significance. Furthermore, in the paired comparison test conducted after 11 days of storage, no significant differences at a 5% level in the aroma of strawberries were found between air- and $CO_2$-stored fruits. However, in the scaling test conducted after 11 days of storage, the average score for the strawberry-like flavor of the $CO_2$ stored

fruit was lower in 'Aromas' (3.5) and 'Selva' (3.2) than in 'Diamante' (6.0), with no significant differences between 'Selva' and 'Aromas' strawberries. 'Diamante' and 'Selva' had a better flavor quality than 'Aromas' strawberries, as indicated by their TA and TSS values and their concentration of organic acids and sugars. Thus, fruit of the former two cultivars (scores 2.3 and 2.4) were preferred by consumers than 'Aromas' (score of 1.5), as indicated by the preference test conducted with >100 consumers. The greater amount of total aroma compounds in 'Diamante', the presence of large amounts of benzaldehyde in 'Selva' fruits and a higher concentration of furanones in both 'Diamante' and 'Selva' may be additional factors influencing consumer preferences.

## 4. Conclusions

Postharvest life and changes in quality attributes and aroma compounds during storage were cultivar dependent. At 5 °C in air, 'Diamante' and 'Selva' showed a longer postharvest life based on appearance than 'Aromas' fruit. The beneficial effect of $CO_2$ during storage was evident by the extension of the postharvest life in the three cultivars and by the increase in firmness in 'Diamante' and 'Selva' with no detectable effects on external color. However, the flavor life was not as long as the postharvest life in both air- and $CO_2$-stored strawberries. Flavor life can be defined as the maximum period of storage during which fruit maintain a similar flavor profile to that present in freshly harvested fruit. Based on the levels of sugars and organic acids, the accumulation of fermentative metabolites and the analysis of aroma profiles during storage, air-stored 'Aromas' strawberries exhibited a shorter flavor life than postharvest life (5 vs. 7 days) while in 'Diamante' and 'Selva' fruits, flavor and postharvest life were the same (9 days). Additional sensory evaluations are needed to confirm this observation.

In $CO_2$-stored 'Aromas' and 'Selva', the flavor life was shorter than the postharvest life (7 vs. 9 and 11 vs. 13 days, respectively). This observation was partially supported by a sensory evaluation,

since the score for strawberry-like flavor was low (3 on a 10-point scale) for both cultivars after 11 days of storage. Finally, since 'Diamante' fruit stored in high $CO_2$ fit the recommended values of TA and TSS for an acceptable flavor, maintained low levels of ethyl esters, preserved the original proportions of ethyl, methyl and other esters, and received a higher score for strawberry-like flavor, we conclude that their flavor life must be similar to their postharvest life (11 days). Again, additional sensory evaluations are needed to confirm this observation.

A more precise definition of flavor life, although more difficult to establish, can be elaborated by considering the recommended levels of flavor components to ensure an acceptable flavor. In strawberries, maximum TA and minimum TSS have been proposed (Mitcham et al., 1996), but values are not available for other flavor components. From correlations between the levels of fermentative metabolites and off-flavor scores, Ke et al. (1991) reported that acceptable maximum levels for acetaldehyde, ethanol and ethyl acetate in 'Selva' strawberries were 8.1, 63 and 23 $\mu l \, l^{-1}$, respectively. However, the presence of fermentative metabolites at low concentrations ($< 50 \, \mu l \, l^{-1}$) is essential to keep a low level of ethyl esters, an active production of methyl and other esters, and a more balanced aroma profile.

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