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Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit

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Abstract

Skin and flesh of 'Selva' strawberries (*Fragaria* x *ananassa* Duch.) stored at 5°C in air or 2 kPa O_2 became darker red and accumulated anthocyanin levels, but these changes were reduced in fruit stored in air + 20 kPa CO_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, and 0.5 kPa O_2 + 20 kPa CO_2 (balance N_2 in all CA treatments). Increasing pH and decreasing titratable acidity in tissues during storage, especially in the internal tissues, were more marked in fruit stored in high CO_2 atmospheres. Since pH affects color expression of the anthocyanin pigment, these changes may contribute to the observed changes in color. Combined citric and malic acid concentrations were higher in the external tissues (10.6 mg g^{-1}) than internal tissues (5.5 mg g^{-1}), and decreases in concentration of both acids were greater in fruit kept in high CO_2 atmospheres. Succinic acid in fruit tissues was present in low concentrations, but usually increased in high CO_2 atmospheres.

Keywords: Anthocyanins; Controlled atmosphere storage; *Fragaria* × *ananassa* (Duch.)

1. Introduction

Anthocyanins, and the factors that affect their synthesis, expression and stability, are responsible for the color of strawberry fruit (*Fragaria* x *ananassa* Duch.). Postharvest color changes and associated increases in anthocyanin synthesis (Kalt et al., 1993; Gil et al., 1997) have been demonstrated in strawberry fruit, but pigment stability has mainly been considered in model systems and juices rather than intact fruit. We observed that storage in elevated CO_2 atmospheres, a commercial treatment for postharvest decay control, induced a paleing or 'bleaching' of the internal flesh color of 'Selva' strawberry (Gil et al., 1997). Further investigation of this phenomenon indicated that two important enzymes in the biosynthetic pathway were adversely affected by elevated CO_2 atmospheres (Holcroft, 1998). While this explains that further synthesis of the anthocyanin pigment is inhibited to a greater or lesser extent, it does not fully explain this 'bleaching' phenomena observed by Gil et al. (1997).

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In mature fruit, cells are compromised predominantly of the vacuole, with the cytoplasm being reduced to a thin layer compressed between the tonoplast and cell wall (Kays, 1997). The vacuole accumulates organic acids, sugars and phenolic compounds including anthocyanin pigments. The accumulation of organic acids results in a buffered solution. Despite this buffering capacity we measured an increase in pH and a reduction in titratable acidity (TA) in the juice of strawberry fruit stored in high CO_2 (Gil et al., 1997; Holcroft, 1998). This increase in pH and decrease in TA has also been observed by Ke et al. (1991) and Wright and Kader (1997).

Since pH has a profound effect on anthocyanin stability and color expression, particularly in an aqueous solution, changes in pH induced by CA treatments could cause significant losses in color. The red flavylium cation (AH^+) remains stable only in acidic conditions (Brouillard et al., 1997). Changes in anthocyanin stability can result from nucleophilic attacks by water molecules at positions 2 and/or 4 on the anthocyanin molecule (Fig. 1) to form the colorless pseudobase, hemiacetal, or carbinol (B). The flavylium form can be restored by acidification. The colorless carbinol can form the yellow chalcone (C), by the opening of the ring structure. While this reaction is reversible, the chalcone form can be irreversibly degraded. As pH increases above 4, the hydroxyl group at 4′ can lose a proton and form the blue quinonoidal base (A). Increases in pH above 7 can result in the loss of a proton from the hydroxyl group at 7 to form a second quinonoidal base (A^-) (Brouillard et al., 1997).

We hypothesized that high CO_2 atmospheres affect pH by dissolution of CO_2 gas and/or by the effect of CA on metabolism of organic acids. Since direct measurement of the pH of large, acidic vacuoles of intact fruit tissues is extremely difficult (Bown, 1985; Kurkdjian and Guern, 1989) we monitored changes in pH, TA and organic acid concentration in extracted juice after fruit were exposed to different atmospheres.

Lewis et al. (1995) investigated the effect of sugar on absorbance of anthocyanins and found that when glucose, sucrose or maltose (all 50%, w/v) were added to a model solution the absorbance increased, although the absorbance maxima remained unchanged. This hyperchromic shift may be caused by a reduction in water activity by high sugar concentrations, causing the equilibrium to favor the flavylium ion. This effect was greater at pH 4 than at pH 2. However, other authors have reported that sugars can have an adverse effect on degradation of the major anthocyanins of strawberry (Sondheimer and Lee, 1949; Daravingas and Cain, 1965). We hypothesized that changes in sugar concentrations in intact fruit tissue may also affect anthocyanin stability.

Our previous research (Gil et al., 1997) has focused on the effect of elevated CO_2 on strawberry fruit color and anthocyanin concentration. Since low O_2 atmospheres, alone or in combination with high CO_2, can have beneficial effects on the postharvest life of strawberries (Ke et al., 1991) we have extended our investigation on anthocyanin metabolism to include these treatments. Li and Kader (1989) found conflicting results of CA treatments on red color. Initially they found that 2 kPa O_2 resulted in higher a^* values, i.e. redder flesh, than air controls. In another experiment they found that 4 days of 0.5 kPa O_2 resulted in lower a^*. Combinations of low O_2 and high CO_2 had the greatest effect on internal or flesh color.

The objectives of this study were to investigate the effects of low O_2 atmospheres alone, or in combination with 20 kPa CO_2 on pH, TA, and organic acid and sugar concentrations, to understand the effects of CA storage on these factors, and their influence on anthocyanin stability and color expression.

2. Materials and methods

2.1. Experimental set-up

'Selva' strawberries were harvested in Watsonville, CA, and transported to the University of California, Davis, in an air-conditioned vehicle. The fruit were sorted on the same day to eliminate all damaged fruit, and selected for uniform size and color. Fifty fruit were placed in 9.5-l jars and

three jars (replicates) were used per treatment. The jars were placed at 5°C and connected to continuous flow (170 ml min^{-1}) of humidified air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2 (balance N_2 in all CA treatments). The gases were

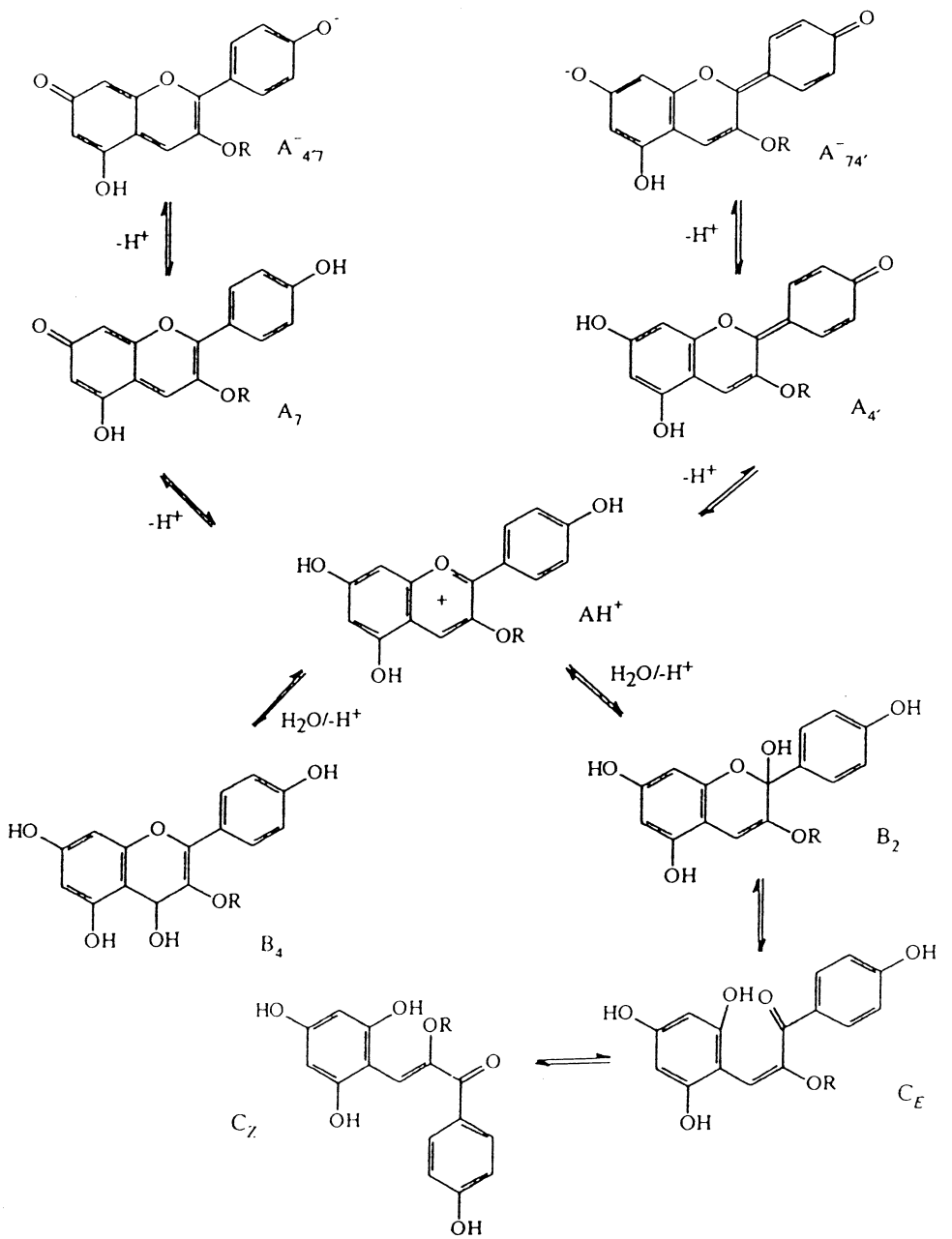

Fig. 1. Structural transformations of pelargonidin 3-glucoside (callistephin), where A and A^- are the blue quinonoidal bases (blue), AH^+ is the red flavylium cation, B_2 and B_4 are the colorless pseudobase/carbinol/hemiacetal and C_E and C_Z are the chalcones (Brouillard et al., 1997).

maintained within 10% of the required concentration (e.g. 20 ± 2 kPa). The composition of the gases was checked regularly with a gas chromatograph equipped with a thermal conductivity detector (model 211; Carle Instruments, Anaheim, CA) or an infrared gas analyzer (model 2000R; Horiba Instruments, Irvine, CA).

2.2. Color

The fruit samples were analyzed initially and after 5 or 10 days of storage at 5°C. Strawberries exhibiting any sign of decay were discarded. Then, external color was measured on opposite sides of the fruit using a Minolta chromameter (model CR-200; Minolta, Ramsey, NJ) which provided CIE L^*, a^* and b^* values. These values were used to calculate chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$), which indicates the intensity or color saturation, and hue angle ($h° = \text{arctangent}[b^*/a^*]$), where $0° =$ red–purple; $90° =$ yellow; $180° =$ bluish-green; and $270° =$ blue (McGuire, 1992). The fruit were sliced lengthwise in half and internal flesh color was measured on both halves.

2.3. Firmness

Fruit firmness was measured on 15 fruit (warmed to 20°C) from each replicate, initially, and after 10 days, by measuring penetration force in Newtons with a UC Firmness Tester (Western Industrial Supply, San Francisco, CA) equipped with a 3-mm probe.

2.4. pH, TA and TSS

Halves of 15 fruit per replicate were separated into internal (pith, bundle zone and about two-thirds of the cortex) and external tissues (epidermis and some cortex), and the juice was analyzed separately for total soluble solids (TSS), pH and TA. TSS was measured using an Abbe refractometer (model 10450; American Optical, Buffalo, NY). A sample of 4 g juice was diluted with 20 ml of distilled water, and pH and TA were measured (titration to pH 8.1) using an automatic titrator fitted with pH meter and an autoburette (PHM85 Precison pH meter and ABU80 autoburette; Radiometer, Copenhagen, Denmark). TA was expressed as percent citric acid.

2.5. Tissue samples for chemical analyses

Halves of 20 strawberries per replicate were frozen in liquid N_2 and kept at -80°C for chemical analyses at a later date. Fifteen halves per replicate were removed from the freezer, allowed to thaw slightly and separated into external and internal tissues as described previously. The separated tissue was immediately refrozen in liquid N_2 before being ground in a stainless steel Oster blender. The powdered tissue was used to prepare anthocyanin, sugar and organic acid extracts.

2.6. Anthocyanin extraction and analysis

The anthocyanins were extracted as specified in Holcroft (1998). The anthocyanins were separated and quantified using an HPLC system (Hewlett-Packard 1050 pump) coupled to a diode array detector (DAD model 1040M, Series II) with an autosampler (1050), operated by HP ChemStation software (version 5). A reverse-phase C_{18} Nucleosil column (150×4.6 mm; particle size 5 μm) with a guard column (Safeguard column holder 5001-CS) was maintained at 40°C. The mobile phase was acidified water (1.32 M formic acid) (A) and acidified methanol (1.32 M formic acid) (B) in a linear gradient where the A:B ratio changed from 82:18 to 65:35 in 12 min, and was held at this ratio for 3 min before returning to the initial ratio. The flow rate was 1 ml min^{-1} and detection was at 510 nm. The injection volume used was 10 μl. Strawberry anthocyanins were identified by retention times and UV spectra. The concentration was quantified by using a commercially available standard of pelargonidin 3-glucose (Pg 3-G, also known as callistephin; Apin Chemicals, UK), which is the most abundant anthocyanin in strawberry. The results were expressed as μg g^{-1} fresh weight of fruit tissue.

2.7. Absorbance

A sample of strawberry juice was diluted 1:5 with a citric acid buffer (0.05 M) that ranged in

pH from 2.6 to 4.4 in increments of pH 0.2. The absorbance was measured on a spectrophotometer (UV-1601; Shimadzu, Japan) at 510 nm. The dilution and measurement were replicated three times at each pH.

2.8. Extraction and analysis of sugars and organic acids

About 25 ml of cold ethanol (90%) was added to 10 g of tissue and blended with a Polytron for 1 min in the dark. The sample was centrifuged at $14\,000 \times g_n$ for 20 min before being filtered. The solution was made up to 50 ml with 80% ethanol (Perez et al., 1997) and stored at -30°C in the dark. An aliquot of 10 ml was taken and dried under a nitrogen stream at <30°C. The residue was dissolved in 2 ml of 0.2 N H_2SO_4 with 0.05% EDTA. The sample was then loaded onto an activated Sep-Pak C_{18} cartridge (Waters) and the eluate collected. The sample was washed through with a further 4 ml of the solution. The eluate was filtered through a 0.45-μm filter and analyzed by HPLC using the system described previously, except that a refractive index (RI) monitor (model 1750; Bio-Rad Laboratories, Hercules, CA) was connected in series with the DAD. The sugars, organic acids and ascorbic acid were separated by a stainless steel ION-300 column (300 × 7.8 mm, 10 μm) (Interaction Chromatography, San Jose, CA) using 0.0085 N H_2SO_4 at a flow rate of 0.4 ml min^{-1} (Perez et al., 1997). Detection was at 210 nm for organic acids and 250 nm for ascorbic acids and the sugars were detected by the RI detector.

2.9. Statistical analysis

Analysis of variance (ANOVA) of the main effects and LSD values with a significance level of 5% were obtained using CoStat Statistical Software (Version 5.01; CoHort Software, Minneapolis, MN). Presented data points are the means of three replications. Chemical assays were performed on each replicate in duplicate or triplicate.

3. Results and discussion

3.1. Visual appearance and firmness

Fruit used in this experiment were produced in Watsonville, CA, in an unseasonally wet spring, consequently the effect of elevated CO_2 treatments in controlling decay was extremely clear. Some of the strawberries stored in air and to a lesser extent in low oxygen, showed some symptoms of *Botrytis* after only 5 days in storage. CO_2-enriched atmospheres were successful in controlling this decay, and retaining flesh firmness close to the initial reading of 6.60 N, after 10 days at 5°C, while fruit stored in air, 2 kPa O_2 and 0.5 kPa O_2 softened considerably over that time period, to 3.95, 4.85 and 5.05 N, respectively.

3.2. Skin and flesh color

The skin (external) color of air-stored strawberries darkened (L^* decreased), became less intensely red (chroma decreased) and was more scarlet than orange–red (hue angle decreased) in comparison to the unstored fruit (Fig. 2). Similar changes, although less extreme, were observed in fruit stored in 2 kPa O_2. The changes in L^* and hue angle were significantly different between air and 2 kPa O_2, and between 2 kPa O_2 and the remainder of the treatments ($P < 0.05$). Nunes et al. (1995) found that strawberries stored in 5 kPa O_2 + 15 kPa CO_2, or 10 kPa O_2 + 20 kPa CO_2 at 4°C were lighter, redder (higher a^*) and had a more intense color (higher chroma) than fruit stored in air. The hue angle was also higher indicating a more orange–red color.

The internal or flesh color of the fruit was much lighter, less intensely red and had higher hue angle than the skin (Fig. 2). Fruit stored in atmospheres containing 20 kPa CO_2 were lighter, less chromatic, and after 5 days had a higher hue angle (more orange red) than fruit stored in air or low O_2 (Fig. 2). The differences in lightness (L^*) between fruit stored in air and 20 kPa CO_2 were significant ($P < 0.05$). The chroma values increased during storage, resulting in higher values for the air and low O_2 treatments (Fig. 2). The hue angle decreased during storage, although the

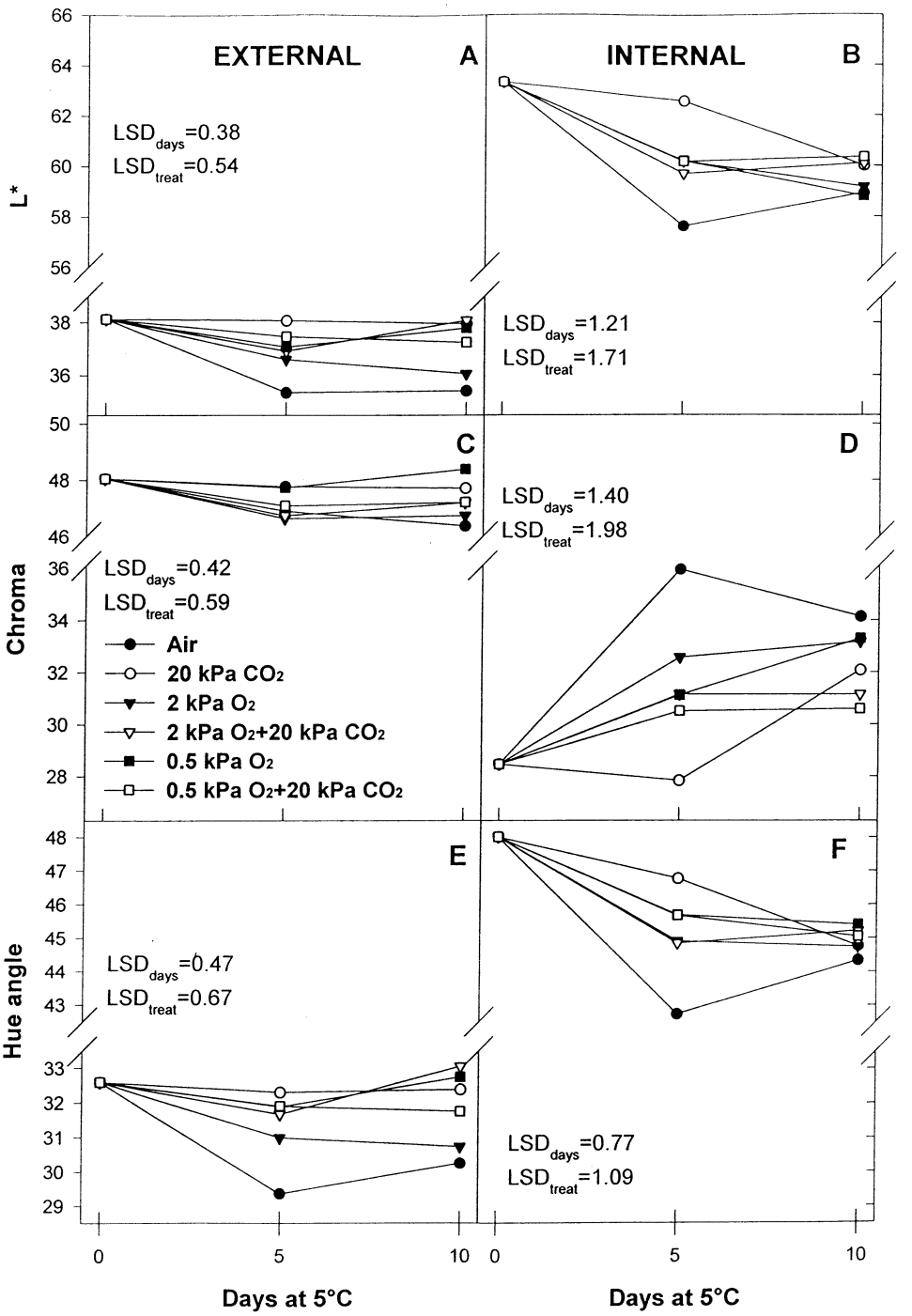

Fig. 2. External (skin) and internal (flesh) color measured as L^* or lightness (A, B), chroma (C, D) and hue angle (E, F) of 'Selva' strawberries stored at 5°C for 5 or 10 days in air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2. LSDs (5%) of main effects are presented.

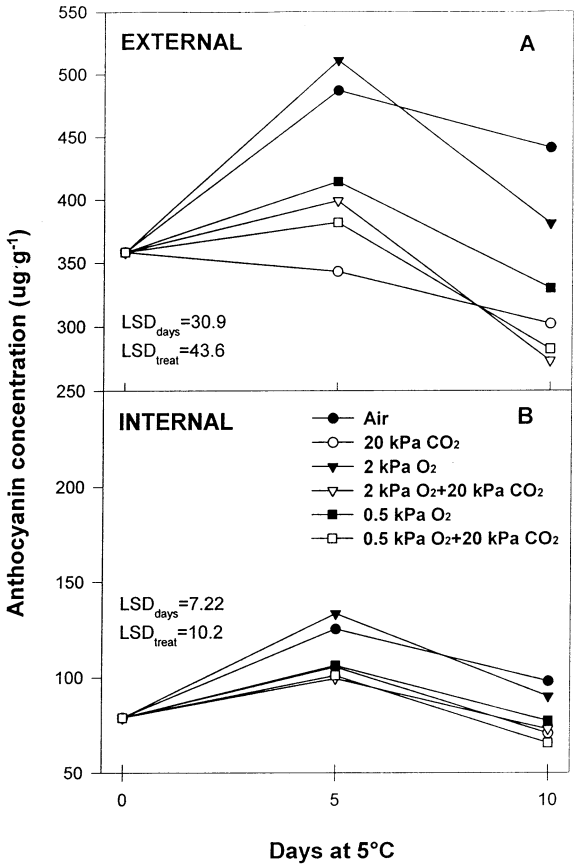

Fig. 3. Total anthocyanin concentration (μg g^{-1}) of external (A) and internal (B) tissues of 'Selva' strawberries stored at 5°C for 5 or 10 days in air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2. LSDs (5%) of main effects are presented. Note the difference in concentration scale between (A) and (B).

differences between treatments were clearer at 5 than at 10 days.

3.3. Anthocyanin concentration

Anthocyanin concentrations were higher in the external tissues (356 μg g^{-1} initially) than in the internal tissues (79 μg g^{-1} initially). Fruit stored in air and 2 kPa O_2 had higher anthocyanin concentrations after 5 and 10 days ($P < 0.05$) (Fig. 3). Fruit stored in 0.5 kPa O_2 tended to have slightly higher anthocyanin concentrations than those kept in CO_2-enriched atmospheres, but this difference was not statistically significant.

Similar changes in anthocyanin concentration were observed in the internal tissues, with an initial increase after 5 days, followed by a decrease in concentration. Similar differences were observed, with fruit stored in air and 2 kPa O_2 having higher pigment concentrations than those in other treatments (Fig. 3).

The apparent decrease in anthocyanin concentration after 10 days at 5°C, was unexpected, because in previous experiments (Gil et al., 1997) the anthocyanin concentration continued to increase in air-stored fruit up to 10 days at 5°C. However, treatments without elevated CO_2 atmospheres showed some decay after 10 days, and decayed fruit were brown and soft; seven fruit (14%) per rep from air, six fruit (12%) per rep from 2 kPa O_2 and one fruit (2%) per rep from 0.5 kPa O_2 were excluded. Fruit that decayed first are usually riper and contain higher anthocyanin concentrations so their exclusion may explain the lower values at 10 days.

External tissues contained, on average, 90% pelargonidin 3-glucose (Pg 3-G), 7% cyanidin 3-glucose (Cy 3-G) and 3% pelargonidin 3-rutinoside (Pg 3-R), initially, and the internal tissues contained 96% Pg 3-G and 4% Pg 3-R. The relative amounts of the different pigments were not affected by the CA treatments. The absence of Cy 3-G in the internal tissues explains the large differences in hue angle between the skin and internal tissues.

Despite the fact that anthocyanin analysis by HPLC is conducted at a low pH to enhance the stability of the pigment, and that low pH favors conversion back to the red flavylium form (Fig. 1), anthocyanin concentrations and visual color were correlated. External anthocyanin concentration was correlated with lightness ($r = -0.64$; $P < 0.001$), chroma ($r = -0.38$; $P < 0.01$) and hue angle ($r = -0.63$; $P < 0.001$) of the skin. Internal anthocyanin concentration was correlated with lightness ($r = -0.40$; $P < 0.01$), chroma ($r = 0.38$; $P < 0.01$) and hue angle ($r = -0.44$; $P < 0.001$) of the flesh.

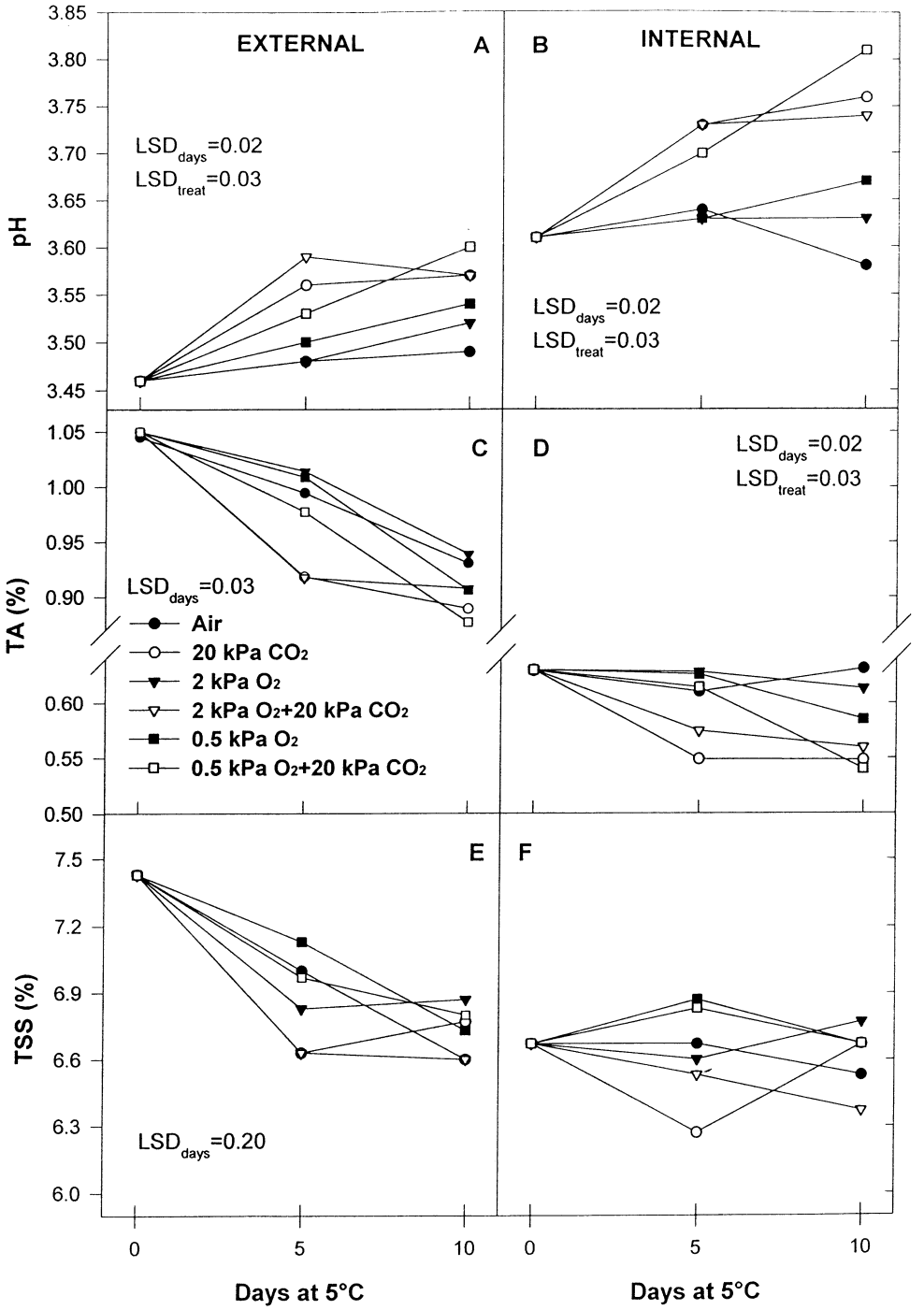

Fig. 4. pH (A, B), titratable acidity (TA) (C, D) and total soluble solids content (TSS) (E, F) of juice from external and internal tissues of 'Selva' strawberries stored at 5°C for 5 or 10 days in air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2. LSDs (5%) of main effects are presented.

3.4. pH and TA

Initially, external tissue pH was slightly lower than the internal tissue pH (3.46 and 3.61, respectively) (Fig. 4). Both external pH and internal pH increased over the 10-day storage period, the increase being greater in elevated CO_2 atmospheres. TA showed a corresponding decrease. TA was considerably lower in internal than external tissues (0.63% compared to 1.05% initially). The difference in pH and TA of internal tissues between fruit stored in air and 20 kPa CO_2 for 10 days was 0.18 and 0.04%, respectively.

Although Li and Kader (1989) did not measure any change in pH or TA in 'Selva' strawberries stored in CA treatments, Ke et al. (1991) measured a slight increase in juice pH of 'Selva' stored in elevated CO_2 atmospheres (20–80 kPa CO_2). pH was unchanged in low O_2 atmospheres (≤ 1 kPa O_2). Nunes et al. (1995) found that 'Chandler' strawberries stored in CA had a slightly higher pH than the air-stored controls. Gil et al. (1997) found that changes in pH and TA tend to be slight when the entire fruit is macerated together, but separating the fruit into external and internal tissues clarified these changes.

Slight changes in pH have a significant effect on the expression of anthocyanin (Fig. 5) since the acidity of the solution affects the ratio between the various forms of the pigments, i.e. red flavylium cation (AH^+), the blue quinonoidal bases (A), the colorless carbinol (B) and pale yellow chalcones (C) (Fig. 1). The pK_a of the reaction between the flavylium ion and the colorless carbinol form of Pg 3-G was found to be 2.98 (Sondheimer, 1953). Therefore, assuming that vacuolar pH is close to juice pH, much of the anthocyanin pigment will have been converted into the colorless carbinol and chalcone, and some irreversible degradation may have taken place. Wrolstad et al. (1970) calculated that a pH change from 3.21 to 3.81 would mean a change in flavylium form from 37 to 13%, and concluded that lowering pH improved the stability of strawberry color more than any other factor. pH of the external tissues was consistently lower than internal tissues (Fig. 4), and this may contribute to the increased stability of the anthocyanin pigment. The pH of the internal tissues was higher and tended to increase with CO_2 treatment. This, combined with the reduced synthesis of anthocyanins under elevated CO_2 atmospheres (Holcroft, 1998) may explain the pale internal flesh color of strawberries stored under these conditions.

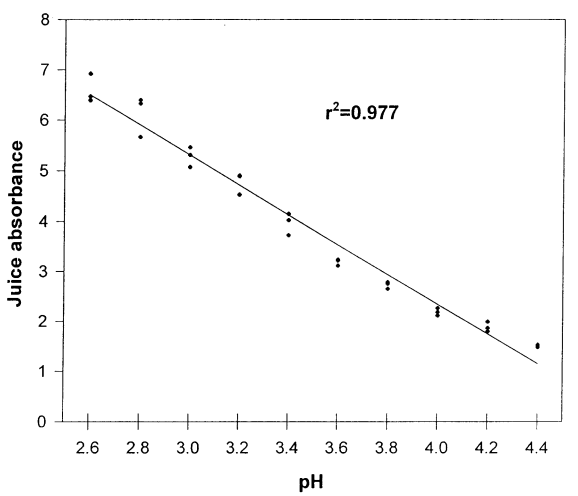

Fig. 5. Changes in absorbance at 500 nm of strawberry juice diluted with a citric acid buffer of increasing pH.

Holcroft (1998) found that fruit stored in 20 kPa CO_2 did not recover their internal color after 3 days in air, indicating that any normalization of pH caused by the return to air did not affect color expression. This suggests that either the effect of dissolved CO_2 is not the primary reason for the pH change, or that if it did cause a pH change in the vacuole, the produced chalcone had already been degraded. Alternatively high CO_2 could affect organic acid metabolism and so influence pH.

Since TA measures the amount of free acid, large differences in TA at about the same pH, such as between the internal and external tissues, indicate the presence of higher organic acid concentrations and consequently a higher buffering capacity. However slight changes in pH in a solution of a given concentration of citric acid affects the ratio between the free acid and the anion, and consequently changes the TA. Because TA is not an accurate measure of total acidity, defined as the sum of acids present as free acids or combined with cations (Ulrich, 1970), direct measurement of organic acid concentration is necessary to accurately reflect changes in total acidity.

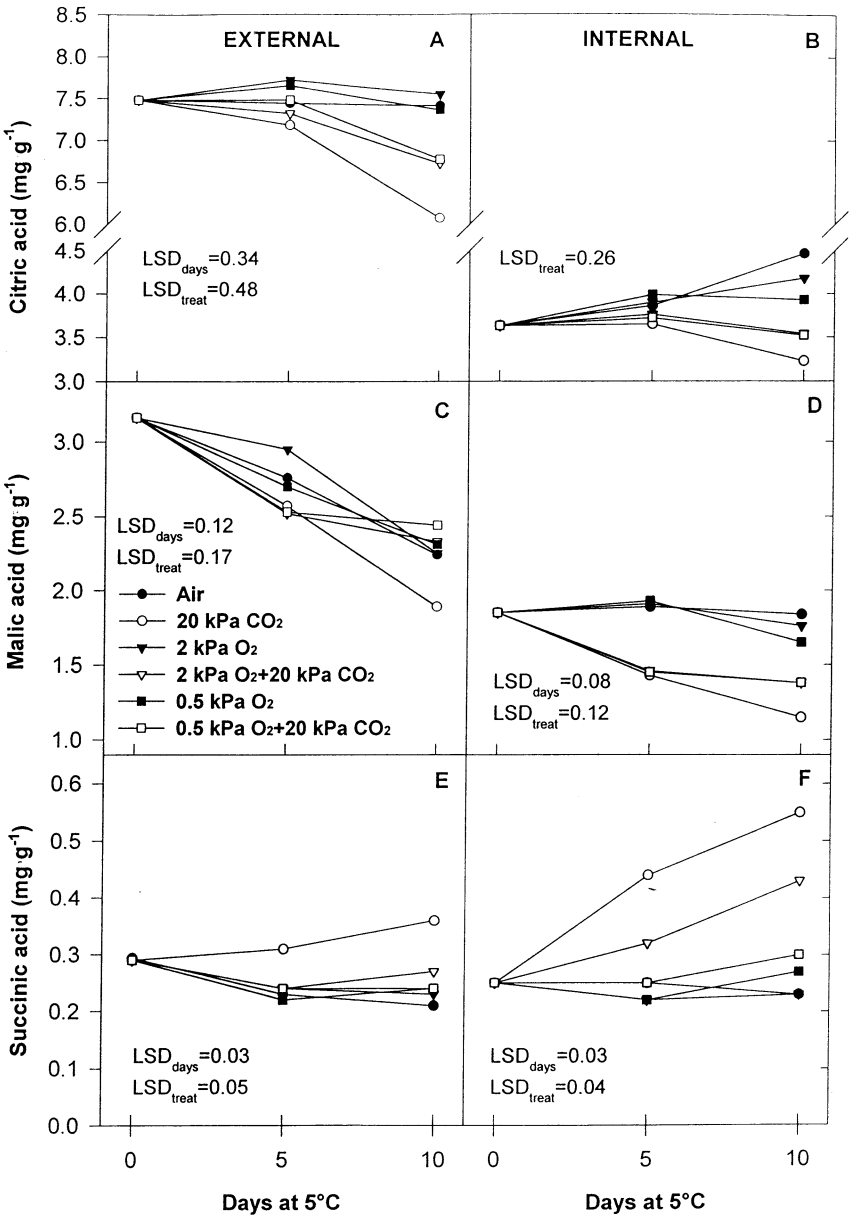

Fig. 6. Concentrations of citric (A, B), malic (C, D) and succinic (E, F) acids of the external and internal tissues of 'Selva' strawberries stored at 5°C for 5 or 10 days in air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2. LSDs (5%) of main effects are presented.

3.5. Organic acid concentration

Citric acid is the most abundant organic acid in strawberry, followed by malic acid. The citric acid concentration in the external tissues was 7.48 mg g^{-1} initially, and declined only in elevated CO_2 atmospheres (Fig. 6). The initial concentration of citric acid in the internal tissues was 3.63 mg g^{-1}. Similar differences between treatments with or without elevated CO_2 atmospheres were noted

after 10 days of storage; however, the citric acid concentration in the internal tissues increased slightly in the treatments with low CO_2 atmospheres.

The malic acid concentration of internal tissues was 1.85 mg g^{-1} initially, compared to 3.16 mg g^{-1} in the external tissues. The concentration of malic acid decreased in both the external and internal tissues of all the fruit, but the decrease was greatest in fruit treated with 20 kPa CO_2 (Fig. 6). Malic acid concentrations in the internal tissues of fruit stored in elevated CO_2 atmospheres were clearly different from the fruit stored in air or low O_2 alone. The changes in malic acid concentration occurred more rapidly than changes in citric acid concentrations and differences were clear after 5 days of storage.

Succinic acid concentrations were low initially but increased during storage in air + 20 kPa CO_2, especially in the internal tissues (0.30 mg g^{-1}) compared with the external tissues (0.07 mg g^{-1}). In the internal tissues the increase was also apparent in fruit stored in low O_2 combined with 20 kPa CO_2, and to a lesser extent 0.5 kPa O_2. An increase in succinic acid in CA-stored strawberries may be a result of inhibition of succinate dehydrogenase activity, as has been shown in other fruits (Williams and Patterson, 1964; Wankier et al., 1970; Frenkel and Patterson, 1973).

Ascorbic acid concentrations were higher in the external tissues (0.29 mg g^{-1}) than in internal tissues (0.12 mg g^{-1}), but were unaffected by the CA treatments. Although strawberries are an important source of ascorbic acid, it is located in the cytoplasm and nucleus (Chinoy, 1984), not in the vacuole where it could interact with anthocyanins. So, while ascorbic acid can have important consequences on anthocyanin stability in processing of fruit or in model systems, its role is less important in intact fruit.

CO_2 hydration and the production of HCO_3^- and H^+ may reduce intracellular pH (Bown, 1985). Lange and Kader (1997) measured a decrease in cytoplasmic pH by ^{31}P NMR spectroscopy in both fruit disks and intact avocado fruit tissue after storage in 20 and 40 kPa CO_2. Vacuolar pH decreased by 0.2 pH units in fruit disks, but did not change in intact fruit. Nanos and Kader (1993) measured a decrease in cytoplasmic pH of 'Bartlett' pears after storage in 0.25 kPa O_2, but they were unable to measure changes in vacuolar pH with ^{31}P NMR spectroscopy since titration curves are insensitive at pH values below 5 (Kurkdjian and Guern, 1989). Siriphanich and Kader (1986) found that cytoplasmic pH of cut lettuce tissue decreased by about 0.4 pH units and the vacuolar pH decreased by 0.2 when stored for 6 days in 15 or 20 kPa CO_2. The pH of lettuce tissue homogenates from the CO_2 treatments, measured in air using a pH meter, had a higher pH than tissue from air-stored controls. Bown (1985) explained a similar observation as an active regulation of pH by cytoplasm that resulted in an increase in pH when returned to air.

Although the increased pH in stored strawberry may be an artifact as in lettuce, acidification of the vacuole in strawberry by dissociation of carbonic acid is unlikely. The accumulation of weak acids such as citric and malic acid means that the vacuole is buffered, as opposed to both lettuce tissue and avocado fruit. While dissociation of carbonic acid in the cytoplasm is likely to occur, since the vacuole is acidic, dissociation of carbonic acid would be slight, and the buffering capacity of the tissue is likely to absorb these changes. Alternatively at the vacuolar pH bicarbonates could form and increase the pH, but these changes should also be buffered.

We bubbled pure CO_2 gas through strawberry juice and did not detect a change in pH (data not shown). Also we measured homogenate pH of strawberry in atmospheres of 20 kPa CO_2, after storage for 2 days in 20 kPa CO_2, and could not discern any changes in pH (data not shown). The same conditions applied to watermelon, which has a homogenate pH of 5.7, resulted in a decrease in pH of 0.1, which rapidly disappeared on return to air. These observations suggest that the response to elevated CO_2 atmospheres can be very different in fruit that have a large, acidic vacuole than in fruit with a higher pH, or in leafy tissues that do not accumulate high concentration of organic acids.

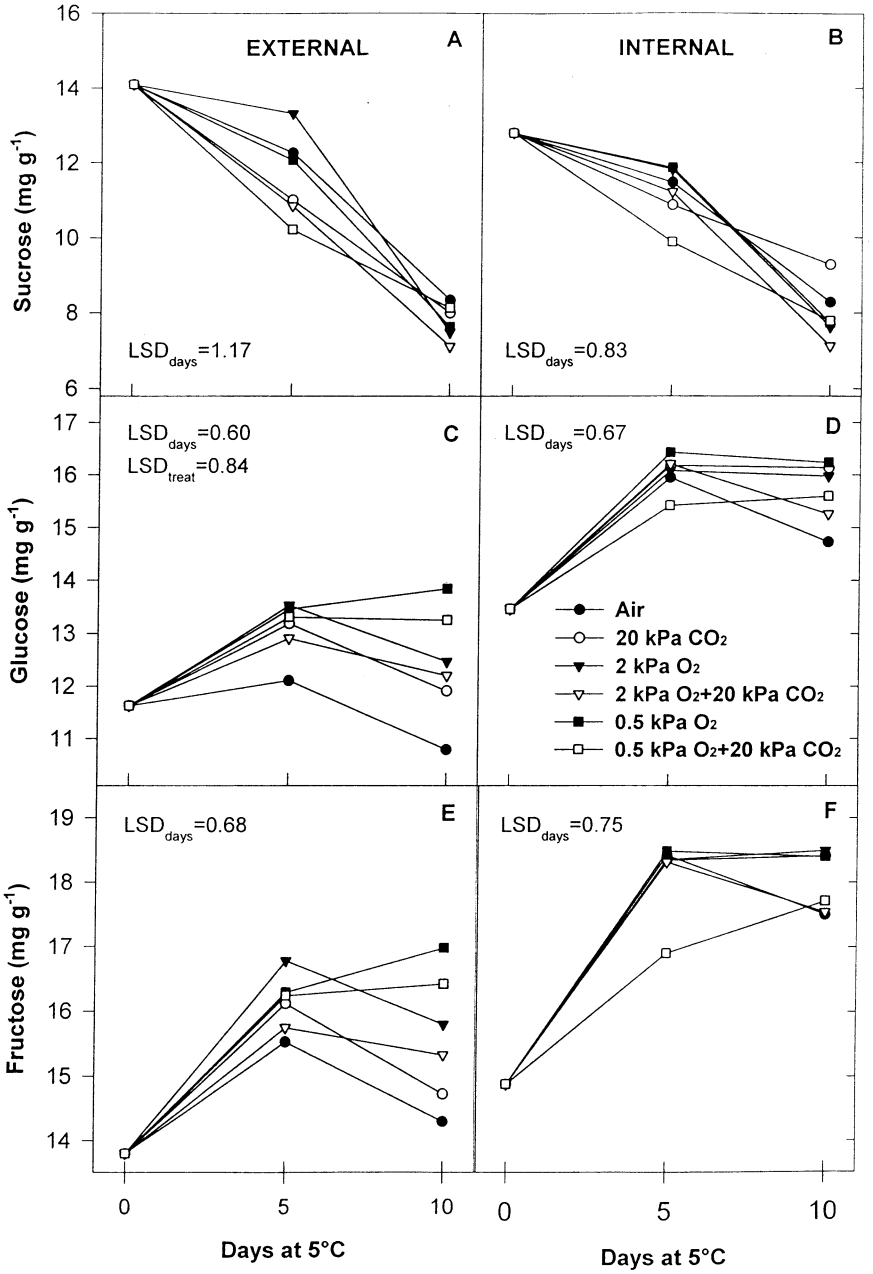

Fig. 7. Sucrose (A, B), glucose (C, D) and fructose (E, F) concentrations from the external and internal tissue from 'Selva' strawberries stored at 5°C for 5 or 10 days in air, air + 20 kPa CO_2, 2 kPa O_2, 2 kPa O_2 + 20 kPa CO_2, 0.5 kPa O_2, or 0.5 kPa O_2 + 20 kPa CO_2. LSDs (5%) of main effects are presented.

3.6. TSS and concentration of sugars

TSS values were slightly higher in the external tissues (7.4%) than in internal tissues (6.7%). TSS in the external tissues decreased significantly after 5 and 10 days, but the changes in the internal tissues were not significant. Comparison of the TSS data and total sugar concentration measured by HPLC showed that these values were not correlated in internal tissues and only loosely

correlated in external tissues ($r = 0.27$; $P < 0.05$). This may be due to the fact that TSS includes sugars, organic acids, soluble pectins, and other constituents.

Changes in the concentrations of sucrose, glucose and fructose were similar in external and internal tissues, with sucrose decreasing, and glucose and fructose increasing, over the 10-day storage period (Fig. 7). The increases in glucose and fructose were less obvious in the air treatment, suggesting that more of the sugar was used in metabolism. Forney and Breen (1986) found that sucrose, glucose and fructose were present at similar concentrations at the time that strawberry fruit began to turn red, but thereafter the sucrose concentration decreased more rapidly than either glucose or fructose. Differences between the CA treatments were not significant but those between the sampling dates were. The one exception is glucose in the external tissues where the air-stored fruit had significantly ($P < 0.05$) lower levels than the other treatments.

The sugar concentrations were not correlated to anthocyanin stability, possibly because the differences between treatments were not large enough to have any significant effect on water activity.

4. Conclusions

Elevated CO_2 treatments, alone or in combination with low O_2, affected strawberry shelf-life. Although low O_2 treatments reduced decay to some extent and delayed softening more than the air controls, the effect was much less than the elevated CO_2 treatments. The 2-kPa O_2 treatment resulted in fruit that were very similar to fruit stored in air, in color, anthocyanin concentration and organic acid metabolism. The response of fruit to 0.5 kPa O_2 was intermediate between the 2 kPa O_2 and high CO_2 treatments.

Most differences occurred between the low CO_2 treatments (air, 2 kPa and 0.5 kPa O_2) and the high-CO_2 atmospheres. Elevated CO_2 atmospheres controlled decay and maintained fruit firmness but adversely affected fruit color, particularly the internal or flesh color. This was associated with an increase in pH, a reduction in malic acid, and to a lesser extent citric acid, and a slight increase in succinic acid. The effects were more noticeable in air + 20 kPa CO_2 (16 kPa O_2) than the combination of low O_2 and 20 kPa CO_2.

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