Changes in Strawberry Anthocyanins and Other Polyphenols in Response to Carbon Dioxide Treatments

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Carbon dioxide-enriched atmospheres are used to reduce the incidence and severity of decay and to extend the postharvest life of strawberries. The influence of CO_2 on the postharvest quality parameters of strawberries, particularly the stability of anthocyanins and other phenolic compounds, was investigated. Freshly harvested strawberries were placed in jars ventilated continuously with air or air enriched with 10%, 20%, or 40% CO_2 at 5 °C for 10 days. Samples were taken initially, and after 5 and 10 days of storage, and color (L^* a^* b^* color space), pH, TA, TSS, and firmness were measured. Anthocyanins and other phenolics were analyzed by HPLC. Elevated CO_2 degraded internal color while air-treated fruit remained red. Internal and external tissues differed in composition and concentration of phenolic compounds. CO_2 had a minimal effect on the anthocyanin content of external tissues but induced a remarkable decrease in anthocyanin content of internal tissues. Factors, such as pH and copigmentation, that could explain this degradation are discussed.

Keywords: *Fragaria × ananassa; controlled atmosphere; quality parameters; anthocyanins; phenolics*

INTRODUCTION

Controlled or modified atmospheres (CA or MA) have been used successfully on strawberries (*Fragaria × ananassa* Duch.). Carbon dioxide-enriched atmospheres (10−20% CO_2 in air) are used to extend the postharvest life of strawberries by reducing respiration rates and ethylene production and action and by retarding softening and incidence and severity of decay (Li and Kader, 1989; Kader, 1991). Higher CO_2 concentrations may have the potential for control of insects, such as thrips and aphids (Aharoni et al., 1979); however, some adverse effects on color have been observed. A change in external color from red to red-purple and watersoaking of the tissue after exposure to 50% or 80% CO_2 for 8 days (Ke et al., 1991) and a reduction in red color intensity of the internal tissue (Kader, 1986) have been reported.

Anthocyanins are water-soluble pigments that are located in the vacuoles and confer a range of colors, from orange to purple. The colorless phenolic compounds can act as copigments and provide protection against UV radiation and insect attack, but they also can be substrates in browning reactions. In addition, phenolic compounds have implications for human health, for example, ellagic acid has been described as an antimutagen and an anticarcinogen and phenolics are reported to reduce coronary heart disease (Maas et al., 1990; Waterhouse, 1995). Thus, it is useful to determine the concentration of these compounds in fruits and the effect of the changes in the atmosphere during storage on the stability of pigments and other phenolic compounds.

There is a scarcity of information about the effects of elevated CO_2 concentrations on phenolic stability. Lin et al. (1989) reported that very high CO_2 concentrations (>73%) as a result of modified atmosphere packaging (MAP) destabilized cyanidin derivatives in the skin of cv. Starkrimson apples. Similar results were found on delphinidin derivatives in pomegranate stored under MAP (Gil et al., 1995b).

Consequently, the objective of this study was to examine the changes in postharvest quality attributes of strawberry and, in particular, changes in anthocyanins and other phenolic compounds during storage under elevated CO_2 atmospheres.

EXPERIMENTAL PROCEDURES

Strawberries (*Fragaria × ananassa* cv. Selva) were harvested in Watsonville, CA, transported to the University of California, Davis, in an air-conditioned car the same day, and stored at 0 °C until preparation for experiments the following morning. The strawberries were sorted to eliminate damaged, poor quality fruit and to obtain a sample that was uniform in size and color. Twenty fruit were selected at random and placed in 2-L jars as one replicate. Three replicates were used per treatment. The jars were placed at 5 °C in air or air enriched with 10, 20, or 40% CO_2. A continuous flow of humidified air at a rate of 100 mL/min was obtained by using flow boards and capillary tubing (Morris, 1969). The composition of atmospheres was verified daily with a Carle gas chromatograph Model 211 using a thermal conductivity detector for CO_2 or an infrared gas analyzer (HORIBA-PIR-2000R), and maintained within 10% of the required concentration (e.g., 10% ±1%).

Fruit samples were analyzed initially and after 5 and 10 days. Ten fruits from each replicate were analyzed for firmness by measuring penetration force in Newtons using a UC Fruit Firmness Tester (Western Industrial Supply Co., San Francisco, CA) equipped with a 3-mm tip (Claypool and Ridley, 1966). External skin color (opposite sides) and internal flesh color (each side of a longitudinally sliced fruit) were measured using a Minolta colorimeter (Minolta, Ramsey, NJ) and expressed as L^* a^* b^* color values (Minolta, 1994). L^* defines the lightness, and a^* and b^* define the red-greenness and blue-yellowness, respectively. Hue angle (H) was calculated as H = arctan b^*/a^* (deg). A sample of 10 fruit halves was wrapped in cheesecloth and squeezed with a hand press, and the clear juice was analyzed for total soluble solids (TSS), pH, and titratable acidity (TA). TSS was measured using an Abbe refractometer (Model 10450). A sample of 4 g of juice was diluted with 20 mL of distilled water, and pH and titratable acidity (TA) were measured using an automatic titrator fitted

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Table 1. Mean (± Standard Deviation) of *L* a* b Color Space *L** Value and Hue Angle, Titratable Acidity (TA), pH, Total Soluble Solids (TSS), and Firmness of Cv. Selva Strawberries Initially and after 5 or 10 Days at 5 °C in Air or CO_2-Enriched Air**

	*L**	hue angle	pH	TA (%)	TSS (%)	firmness (N)
initial	36.4 ± 1.2	28.7 ± 1.8	3.51 ± 0.04	0.75 ± 0.04	7.6 ± 0.0	15.4 ± 1.4
5 days						
air	35.8 ± 1.8	28.6 ± 0.4	3.48 ± 0.01	0.73 ± 0.02	6.7 ± 0.2	4.2 ± 0.3
10% CO_2	36.8 ± 0.3	30.2 ± 1.0	3.53 ± 0.04	0.75 ± 0.05	7.3 ± 0.4	4.5 ± 0.5
20% CO_2	37.7 ± 0.9	30.4 ± 0.8	3.54 ± 0.03	0.76 ± 0.05	7.4 ± 0.5	5.0 ± 0.4
40% CO_2	38.0 ± 0.7	30.7 ± 0.3	3.64 ± 0.03	0.69 ± 0.04	7.3 ± 0.3	4.9 ± 0.4
10 days						
air	32.2 ± 0.1	26.3 ± 0.2	3.50 ± 0.03	0.72 ± 0.05	6.7 ± 0.3	4.1 ± 0.1
10% CO_2	33.6 ± 0.2	27.2 ± 1.0	3.48 ± 0.03	0.76 ± 0.03	6.5 ± 0.3	4.6 ± 0.1
20% CO_2	35.3 ± 0.9	27.7 ± 1.5	3.54 ± 0.02	0.74 ± 0.03	6.8 ± 0.2	5.0 ± 0.6
40% CO_2	34.7 ± 1.5	25.7 ± 2.1	3.71 ± 0.06	0.65 ± 0.05	6.9 ± 0.4	3.8 ± 0.4
LSD (5%)	1.4	1.4	0.04	0.06	0.4	0.9

with a PHM85 Precision pH meter and an ABU80 autoburette. The remaining 10 fruit from each replicate were frozen in liquid nitrogen and transferred to −80 °C for subsequent phenolic analysis. At 0 and 10 days, fruit remaining from the physical and chemical analyses were dissected into internal (pith, bundle zone, and some cortex) and external tissue (epidermis and some cortex) and frozen under the same conditions as the whole fruit. The pH and TA of the internal and external flesh were measured from a puree sample.

Due to the high pectin content of fresh strawberry fruit, phenolic extraction with methanol yielded extracts with a high pectin content making them difficult to handle, concentrate, and filter for HPLC analysis. Extraction with acetone has been used for flavonoid extraction from pectin rich fruits, e.g., citrus (Chatterjee and Chatterjee, 1988), and so this extraction system was used for strawberries. In fact, the acetone extraction was more efficient in this case than the classical methanol extraction. The fruit were blended to a smooth puree in an Oster blender. Samples of 5 g were mixed with 15 mL of acetone using a Polytron for 1 min before being filtered on a Buchner funnel through glass wool and Whatman No. 3 filter paper under vacuum. The filtrate was evaporated under a nitrogen stream in a water bath at 35 °C. The concentrated sample was dissolved in 5 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters), previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% aqueous formic acid. The anthocyanins and other phenolics were then recovered with 1.8 mL of methanol containing 3% formic acid. The methanolic extract was filtered through a 0.45-μm filter and 20 μL was analyzed by HPLC. Two extracts were done from each replicate.

The phenolic compounds were separated using an HPLC system (Hewlett Packard 1050 pump) coupled to a photodiode array detector (Series 1040M, Series II) with an autosampler (Series 1050), operated by HP ChemStation software. A reverse phase C_{18} Nucleosil column (150 × 4.6 mm; particle size 5 μm) with a guard column (Safeguard holder 5001-CS) was maintained at 40 °C. The mobile phase was acidified water (2.5% formic acid) (A) and acidified methanol (2.5% formic acid) (B) in a linear gradient from 15 to 30% B in the first 15 min, followed by an isocratic mixture for 5 min, then a linear gradient from 30 to 80% B for 5 min, an isocratic mixture for 2 min before returning to the initial conditions. The flow rate was 1 mL/min and detection was performed at 280, 320, 350, and 510 nm. Scanning between 240 and 550 nm was done every 0.32 s for the entire chromatogram. Retention times and spectra were compared to pure standards.

The strawberry extract was separated by cellulose thin layer chromatography (TLC) (Analtech, Inc., 20 × 20 cm, 250 μm thick) using water as a mobile phase. The different chromatographic bands were eluted from the cellulose with acidified MeOH (3% formic acid) and then hydrolyzed by the addition of 20% (volume added to the extract) of 2 N HCl for 30 min for glucosides and 2 h for glucuronides, and the aglycons were identified by HPLC under the same conditions as described above.

Phenolic standards, e.g., cyanidin 3-glucoside (Apin Chemicals Ltd., U,K,), *p*-coumaric acid, ellagic acid, kaempferol, and quercetin standards (Sigma, St. Louis, MO) were injected three times at two concentrations (0.1 and 0.05 mg/mL) for external standard quantification. Anthocyanins were quantified using cyanidin 3-glucoside as a standard, flavonols were quantified using quercetin 3-glucoside, and *p*-coumaroylglucose was quantified using *p*-coumaric acid; for ellagic acid, the authentic standard was used. The results were expressed as micrograms per gram fruit tissue.

RESULTS AND DISCUSSION

External and Internal Color. Skin color was not markedly affected by the different CO_2 treatments. There was a slight decrease in the external *L* a* b** color '*L*' value especially in air (Table 1). A decrease in the external hue angle was observed, particularly for fruit held in air + 40% CO_2 (Table 1). The fruit became darker with storage as ripening progressed, in accordance with previous reports (Kalt et al., 1993; Miszczak et al., 1995). Therefore, moderate carbon dioxide levels had little effect on strawberry external color and only high levels affected skin color. This is in agreement with previous reports (Li and Kader, 1989) where elevated CO_2 levels (10%, 15%, or 20%) retarded color development.

When internal flesh color was evaluated, marked changes were observed under CO_2 treatments as compared with air (Figure 1). Internal *L** value increased substantially in all CO_2 treatments but not in air. Both *a** and *b** values were decreased by CO_2. No differences were noted among the three CO_2 concentrations tested. Therefore, compared with the initial sample, CO_2 treatments degraded internal color but air treatment retained it. Smith and Skog (1992) measured the color of a blended strawberry sample and found no effect of storage under 15% CO_2 on color. We also used this method and found some differences, but these were not as clear as the color measurement on the internal flesh of the fruit.

pH, TA, TSS, and Firmness. The pH of the strawberry juice was slightly higher after storage under 40% CO_2, and there was a corresponding decrease in TA. But there were no significant variations with the other treatments (Table 1). Ke et al. (1991) found that CO_2 treatments (20, 50, and 80% CO_2) caused an increase in pH after 10 days at both 0 and 5 °C. Soluble solids (TSS) slightly decreased after 5 days in air and after 10 days in CO_2, but there were no differences between treatments (Table 1). The firmness of the berries decreased from 15.4 to 4.2 N after only 5 days of storage, and there were no further changes after 10 days. There were no significant differences between CO_2 treatments

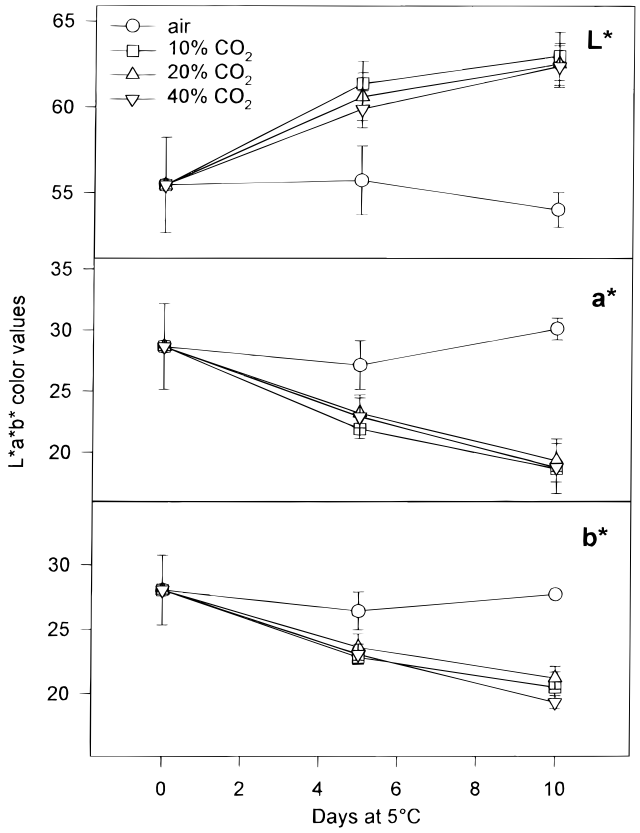

Figure 1. Effect of CO_2 on color of Selva strawberries stored at 5 °C for 5 or 10 days in air or CO_2-enriched atmospheres.

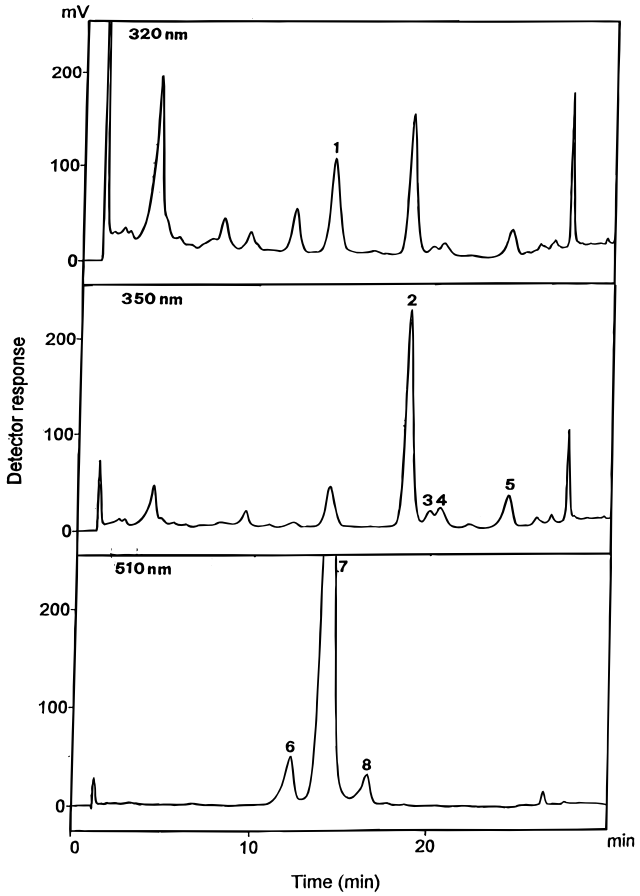

Figure 2. HPLC chromatograms of strawberry anthocyanins and other phenols: (1) *p*-coumaroylglucose, (2) quercetin 3-glucoside + quercetin 3-glucuronide, (3) ellagic acid, (4) ellagic acid derivative with the same UV spectrum as ellagic acid, (5) kaempferol 3-glucoside + kaempferol 3-glucuronide, (6) cyanidin 3-glucoside, (7) pelargonidin 3-glucoside, and (8) pelargonidin 3-rutinoside.

and air in maintaining firmness (Table 1). del Rio et al. (1987) observed a similar decrease in firmness after CO_2 storage when compared to initial for cv. Douglas strawberries. These results differ from previously published data where CO_2 retarded softening (Li and Kader, 1989; Smith, 1992; Smith and Skog, 1992; Larsen and Watkins, 1995a,b).

Phenolic Compounds. The HPLC analysis of strawberry extracts showed that, in addition to the anthocyanins, other phenolic compounds were present in significant amounts (Figure 2). Compounds with characteristic spectra of a *p*-coumaric acid derivative (Mosel and Hermann, 1974), ellagic acid (Rommel and Wrolstad, 1993), quercetin 3-*O*-glycoside, and kaempferol 3-*O*-glycoside (Mabry et al., 1970) were detected. Previously, the occurrence of *p*-coumaroylglucose, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide was reported in strawberries (Macheix et al., 1990), and ellagic acid was also described as an important phenolic constituent of this fruit (Rommel and Wrolstad, 1993). Ellagic acid was readily identified by comparison with an authentic standard. Under the chromatographic conditions used in the HPLC analysis, a single peak of quercetin derivatives and a single peak of kaempferol derivatives were resolved (Figure 2). To determine if glucuronides and glucosides were present under the same chromatographic peak, the extract was separated by TLC using water as solvent. Under these conditions the glucuronides, which are often difficult to resolve from glucosides in most chromatographic systems, move close to the solvent front, while the glucosides remain at the origin (Markham, 1982). In cv. Selva strawberry extracts, both glucuronides and glucosides were detected after TLC. The glucuronide band was eluted and analyzed by HPLC, and quercetin and kaempferol glucuronides were detected. After hydrolysis, the corresponding aglycons were identified by comparisons with authentic standards. The glucoside fraction also contained quercetin and kaempferol glucosides. For the purpose of this work, quercetin glucosides and glucuronides were quantified together, as were kaempferol glucosides and glucuronides. During the TLC separation of glucuronides and glucosides, another UV-absorbing fraction was detected. After elution and HPLC analysis, it was identified as the *p*-coumaric derivative. This was confirmed after acid hydrolysis, which yielded *p*-coumaric acid and glucose.

The concentration of both quercetin and kaempferol derivatives was higher after 10 days of storage. After the initial increase, they remained comparatively stable (Table 2). Ellagic acid significantly increased, although the increase was delayed at higher CO_2 concentrations. This increase could be explained as a degradation of ellagitannins. *p*-Coumaroylglucose increased during storage, especially in those fruit stored under low CO_2. Degradation of *p*-coumaroylglucose appears to be greater after 10 days of air + 40% CO_2, possibly due to the weak ester linkage.

Anthocyanin Analysis. Selva strawberries contained three anthocyanins (Figure 2). Cyanidin 3-glucoside and pelargonidin 3-glucoside (main component) were identified by retention times, by UV−vis spectra recorded with a diode array detector, and by comparison with pomegranate extracts containing these pigments

Table 2. Mean (± Standard Deviations) of Quercetin and Kaempferol Derivatives, Ellagic Acid, and *p*-Coumaroylglucose (μg/g) in Whole Strawberry Fruit Initially and after 5 or 10 Days at 5 °C in Air or CO_2-Enriched Air

	quercetin deriv	kaempferol deriv	ellagic acid	*p*-coumaroyl-glucose
initial	40.1 ± 6.6	13.7 ± 2.5	19.9 ± 2.7	9.8 ± 4.1
5 days				
air	44.1 ± 6.1	15.8 ± 1.9	26.8 ± 2.7	16.7 ± 2.9
10% CO_2	47.5 ± 3.4	15.2 ± 0.2	22.2 ± 0.3	12.6 ± 2.9
20% CO_2	42.7 ± 2.3	15.2 ± 1.4	22.3 ± 0.3	13.9 ± 3.2
40% CO_2	44.4 ± 1.9	14.5 ± 1.7	21.9 ± 3.5	10.6 ± 1.4
10 days				
air	46.4 ± 3.0	16.0 ± 1.4	26.6 ± 0.7	14.8 ± 5.0
10% CO_2	42.8 ± 2.5	15.1 ± 0.8	25.1 ± 2.3	14.5 ± 3.3
20% CO_2	45.4 ± 1.0	15.6 ± 0.2	27.8 ± 0.7	10.5 ± 2.6
40% CO_2	49.1 ± 1.0	16.6 ± 0.9	28.5 ± 1.1	7.0 ± 1.0
LSD (5%)	6.1	2.4	3.3	5.3

Table 3. Mean (± Standard Deviations) of Total Anthocyanins (μg/g) in Whole, External, and Internal Tissue of Cv. Selva Strawberry Fruit Initially and after 5 or 10 Days at 5 °C in Air or CO_2-Enriched Air

	whole fruit	external tissue	internal tissue
initial	120.2 ± 19.6	195.3 ± 11.5	55.1 ± 6.1
5 days			
air	153.5 ± 12.4		
10% CO_2	115.3 ± 21.8		
20% CO_2	125.0 ± 12.2		
40% CO_2	115.4 ± 16.4		
10 days			
air	142.4 ± 38.6	256.7 ± 10.6	76.9 ± 8.5
10% CO_2	138.4 ± 21.9	173.4 ± 25.2	43.2 ± 3.6
20% CO_2	118.5 ± 18.7	179.1 ± 40.8	36.1 ± 11.2
40% CO_2	113.7 ± 13.5	166.0 ± 11.8	30.3 ± 7.3
LSD (5%)	34.8	41.6	13.9

(Gil et al., 1995a). The third peak, eluting after pelargonidin 3-glucoside, had an identical UV−vis spectrum to pelargonidin 3-glucoside. Bakker et al. (1994) identified a peak eluting under similar conditions as pelargonidin 3-rutinoside. Initial anthocyanin content was 120.2 μg/g (Table 3), and there were no significant differences between this initial value and the values obtained from stored fruits. The total anthocyanin amount of the whole fruit did not reflect the visible changes in flesh color. Fruits stored in air showed higher pigment content, especially after 5 days, but it was not significantly different from the rest of the treatments. The anthocyanin content of CO_2-treated fruit was reduced as compared with air-stored fruit. The variation among replicates was high, but the results indicated that an increase in anthocyanin content took place during storage, especially in air where metabolism is not affected as much as in CO_2-enriched atmospheres.

External and Internal Tissue Analysis. Anthocyanin analysis of the whole fruit did not explain the difference in the flesh color of fruit stored under air + CO_2 as compared to fruit stored in air, where the color was maintained. Consequently the internal and external tissues were analyzed separately for anthocyanin content and pH. External and internal anthocyanin contents were significantly different in fruits stored in air as compared with the initial or with those stored under CO_2-enriched atmospheres (Table 3). Fruit storage under air increased anthocyanin content presumably by promoting anthocyanin synthesis, in both internal and external tissues. No differences in external anthocyanin content between fruit stored under CO_2-enriched atmospheres and initial fruit were observed.

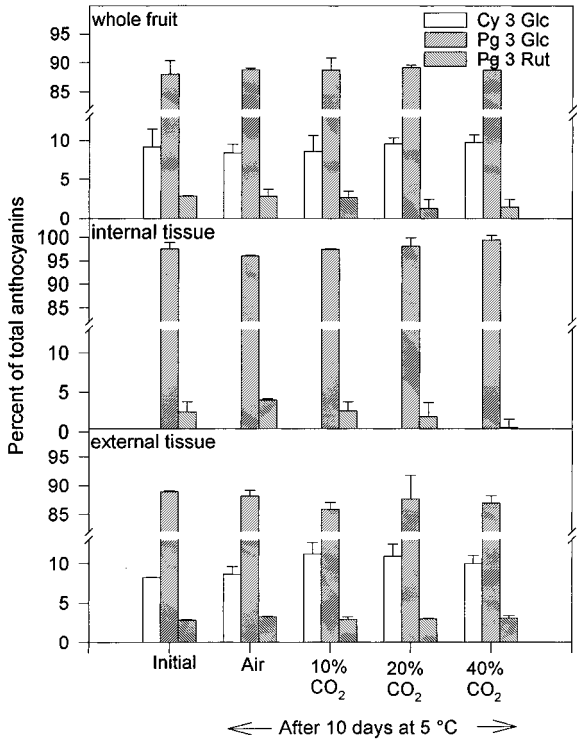

Figure 3. Anthocyanin composition of Selva strawberries stored at 5 °C for 10 days in air or CO_2-enriched atmospheres.

Table 4. Mean (± Standard Deviations) of Quercetin and Kaempferol Derivatives, Ellagic Acid, and *p*-Coumaroylglucose (μg/g) in External and Internal Cv. Selva Strawberry Fruit Tissue Initially and after 10 Days at 5 °C in Air or CO_2-Enriched Air

	quercetin deriv	kaempferol deriv	ellagic acid	*p*-coumaroyl-glucose
		External		
initial	63.8 ± 5.6	21.8 ± 2.3	33.3 ± 3.6	15.0 ± 5.2
air	80.3 ± 14.8	27.9 ± 3.6	52.3 ± 5.4	12.4 ± 2.0
10% CO_2	83.8 ± 14.5	26.1 ± 4.5	44.8 ± 5.2	7.8 ± 2.6
20% CO_2	79.3 ± 16.6	26.8 ±4.3	46.6 ± 3.6	8.8 ± 2.0
40% CO_2	76.3 ± 7.2	25.5 ± 1.6	48.2 ± 5.8	9.7 ± 3.4
LSD (5%)	22.5	6.2	8.7	5.9
		Internal		
initial	3.3 ± 2.1	3.3 ± 0.6	8.4 ± 1.1	17.2 ± 5.1
air	2.2 ± 0.3	6.7 ± 0.5	9.3 ± 2.2	16.8 ± 3.1
10% CO_2	3.4 ± 2.8	3.1 ± 0.8	9.4 ± 1.7	10.5 ± 3.1
20% CO_2	3.1 ± 0.9	3.1 ± 0.5	8.0 ± 1.1	14.7 ± 1.4
40% CO_2	2.6 ± 0.8	2.8 ± 0.4	8.7 ± 1.5	12.2 ± 4.1
LSD (5%)	3.0	1.0	2.8	6.4

However, a remarkable decrease in internal anthocyanin content was observed, particularly at 20 and 40% CO_2.

A comparison of the different HPLC anthocyanin profiles showed that there were no differences in the percentage of each anthocyanin if the analysis was done with the whole fruit (Figure 3). However, very different profiles were found for external and internal tissues. The internal sample did not have cyanidin 3-glucoside, which confers a redder color than the orange pelargonidin derivatives (Figure 3). As CO_2 levels increased, the concentration of pelargonidin glycosides in the internal tissue decreased, contrary to the increase observed in air. Cyanidin 3-glucoside, located in the external tissue, appeared to be fairly resistant to degradation (Figure 3). There was an increase in percentage of this pigment after 10 days of storage in 10 and 20% CO_2, which explains the deeper red color. These results suggest

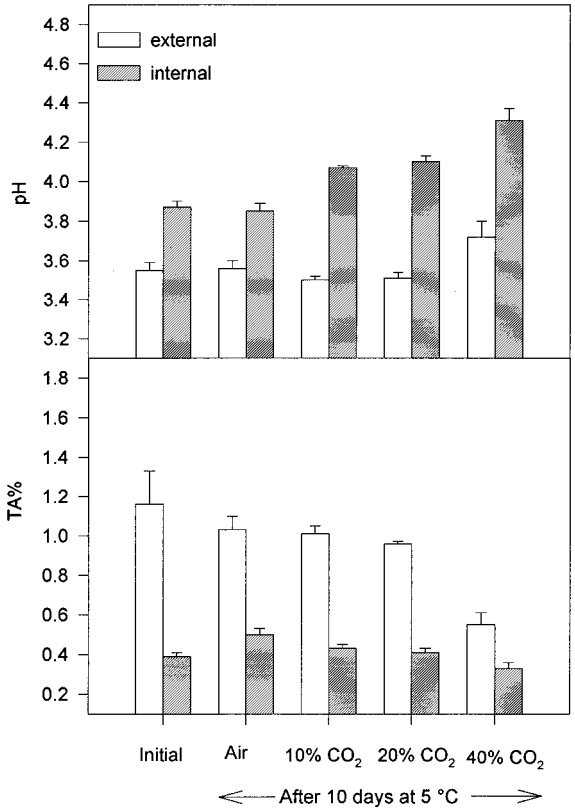

Figure 4. Effect of CO_2 on pH and titratable acidity (TA) of Selva strawberries kept at 5 °C for 5 or 10 days in air or CO_2-enriched atmospheres.

that most of the anthocyanin degradation takes place in the internal tissue.

External tissue had higher concentrations of ellagic acid and quercetin and kaempferol derivatives (Table 4). *p*-Coumaroylglucose was present in similar concentrations in the external and internal tissues. The accumulation of phenolic compounds in epidermal tissue is consistent with the proposed protective roles of these substances as antimicrobial metabolites and as UV screens (Harborne, 1982).

It is difficult to explain the reduction in color in the internal tissues of strawberries stored under CO_2, and factors such as copigmentation, pH, and anthocyanin metabolism may play a significant role in the expression of color in strawberries. The most efficient copigments are flavonols (Mazza and Miniati, 1993), and they are almost exclusively located in the external tissues of strawberry (Table 4). The observed anthocyanin stability in the external tissue could be explained by intermolecular copigmentation with flavonols and other phenolics, while anthocyanins in internal tissues, where flavonols are present in very low concentrations, would be more susceptible to degradation. Perhaps this increased susceptibility to degradation in combination with a reduction in anthocyanin synthesis in strawberries exposed to 10%−40% CO_2 could explain the 43−61% decrease in anthocyanin concentration in the internal tissue.

The color and stability of anthocyanins are known to be influenced by pH. As pH increases, color fades. At pH 4−6, most anthocyanins appear colorless (Brouillard, 1982; Mazza and Miniati, 1993). Vacuolar pH is typically lower than overall pH (Moskowitz and Hrazdina, 1981), and this contributes to the expression of color. In our study, the corresponding pH measurement on the internal and external tissue (Figure 4) showed that pH increased with CO_2 treatments and was higher in the internal tissue. The effect of CO_2 on pH is contradictory since both increases and decreases in pH have been reported. Siriphanich and Kader (1986) found that when lettuce was maintained under 15% CO_2, pH was lower, but when transferred to air an increase in pH was observed. They also observed a corresponding decrease in TA. In the present work, a reduction in TA in the external tissue and a slight increase in pH was observed (Figure 4). However, TA of the internal tissue was lower, and this may confer a lower buffering capacity against pH changes, since pH increased by 0.44.

In conclusion, the advantages of elevated CO_2 in maintaining shelf life and postharvest quality of strawberry have been reported, but adverse effects of 20% and 40% CO_2 on anthocyanin stability, particularly in the interior of the fruit, was noted. Several suggestions to explain these changes were discussed, but further research on copigmentation and the effect of CO_2 on both vacuolar pH and stability of anthocyanins is necessary.

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