CHANGES IN 'DELICIOUS' APPLE BROWNING AND SOFTENING DURING CONTROLLED ATMOSPHERE STORAGE

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ABSTRACT

High CO_2 conditions offered no advantage over normal CA storage in terms of reduced 'Delicious' apple browning or softening during a 28 week storage period. After approximately 7-14 weeks in CA storage, 'Delicious' apples showed significant losses in PPO activity, browning tendency and firmness. Electrolyte leakage reached a maximum at 11.5 weeks and declined, while total phenolics remained fairly constant. The changes observed may indicate a loss in membrane integrity and decompartmentalization after 7-14 weeks of storage, which may in turn allow for increased enzymatic browning. Polygalacturonase activity was only detectable at harvest. Holding apples for additional time in air following removal from CA storage appears to accelerate changes in these characteristics.

INTRODUCTION

Controlled atmosphere (CA) storage, which utilizes low O_2 and high CO_2 concentrations, is commonly used for apples because it slows respiration and

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maintains quality for longer periods of time. High CO_2 concentrations (>7%) have also been used as pre-storage treatments when cooling facilities are limited, and some investigators (Couey and Olson 1977; Porritt and Meheriuk 1977; Mattus 1982) report improvements in firmness as a result of this treatment.

Although the use of CA storage enjoys wide commercial practice, little is known about the effects of reduced O_2 and elevated CO_2 on biochemical and physiological reactions which take place in apple tissue after harvest, in particular, enzymatic browning, membrane breakdown and loss of cellular integrity. Controlled atmospheres may affect cellular integrity and result in decompartmentalization and interaction of polyphenol oxidase (PPO) and its phenolic substrates. Various investigators (Buescher and Henderson 1977; Ong 1987; Siriphanich and Kader 1985) have found that levels of CO_2 above 5% inhibit polyphenol oxidase, the enzyme responsible for enzymatic browning. Siriphanich and Kader (1985) also reported that, in lettuce, an atmosphere of air + 15% CO_2 inhibited production of polyphenolic compounds, the substrates for the browning reaction. However, even though use of elevated CO_2 concentrations appears to reduce PPO activity and phenolics content, flesh browning was found to be greater in pears (Ong 1987).

Apple softening results from degradation of cell wall polysaccharides, thought to be enzyme catalyzed, and the loss of membrane and organelle integrity during the course of normal senescence. Although an exo-polygalacturonase (PG) has been purified from apples (Bartley 1978), an endo-PG has not been found in apples (Pilnik and Voragen 1970) and pectin esterase seems to play a minor role in cell wall softening (Bartley and Knee 1982). The activities of other enzymes such as ß-galactosidase and endo-ß-1,4-glucanase are also quite low in apples. Therefore, apple softening through the degradation of cell wall polysaccharides is an unresolved issue. Other indicators of changes in apple tissue texture, such as electrolyte leakage and firmness are often used to assess softening (Ferguson and Watkins 1981; Couey and Olson 1977).

The objectives of this study were to determine whether an elevated, varying CO_2 storage environment offered advantages over normal CA conditions in terms of reduced apple browning and softening. A preliminary study by Liu and Pan (1989) indicated that use of such a storage regime may result in improved 'Delicious' apple texture, however results regarding browning were inconclusive. In addition, in order to mimic a typical marketing situation, the degree of additional browning and softening which occurred after holding apples one week at 18°C were examined.

MATERIALS AND METHODS

Raw Materials and Storage Conditions

'Delicious' apples are harvested from Cornell University Orchards and stored using a flow-through simulated CA storage system, as described by Liu and

Samelson (1986). Five gallon (19 L) glass jars, each containing 45 apples from the same tree, were stored at 0° ± 1°C. Three replicate jars were sampled per treatment per storage time. Gas mixtures containing the appropriate amounts of O_2, CO_2, and N_2 were mixed and humidified as described previously (Liu and Samelson 1986) and flowed at a rate of 200 mL per minute.

Apples were stored under normal CA and elevated, varying CO_2 conditions, modeled after Liu and Pan (1989), during 1987/88 and 1988/89. Normal CA gas mixtures were composed of 2% O_2, 3% CO_2, and 95% N_2 while the elevated, varying CO_2 condition changed with time in storage as described below:

Days 1-40 (0-5.5 wks)	2.5% O_2	12.0% CO_2	85.5% N_2
Days 41-70 (5.5-10 wks)	4.0% O_2	8.0% CO_2	88.0% N2
Days 71-160 (10-22.5 wks)	6.0% O_2	10.0% CO2	84.0% N2
Days 160-180 (22.5-28 wks)	5.0% O2	9.0% CO2	86.0% N2

This storage regime was recommended by horticulturalists from the Shanxi Fruit Research Institute of northwest China, who collaborated on the project.

Apples were removed from storage after 7, 14, 21 and 28 weeks in the first year study and 7, 11.5, 14, 21 and 28 weeks in the second year study. A portion of the apples was analyzed immediately, while the other portion was analyzed after being kept in regular air storage at 18°C for 7 days. The latter treatment was representative of a simulated marketing condition.

Total Phenols Extraction and Assay

The phenolics in apples were extracted and assayed using a modification of the procedure described by Weurman and Swain (1955). Fifty gram samples of peeled apple were homogenized with 200 mL of 70% (v/v) ethanol for three minutes in a Waring Blendor and the homogenate was centrifuged at 760xg (Sorvall RC-5B Refrigerated Superspeed Centrifuge) for 10 min. The supernatant was saved, the pellet re-extracted with 100 mL of 60% (v/v) ethanol and centrifuged at 760xg for 10 min. The pellet was discarded, the supernatant combined with the earlier one and filtered. Five hundred µL of filtrate were diluted with 10.5 mL of water and 2.0 mL of Folin and Ciocalteau Phenol Reagent (Sigma Chemical Co.) were added for color development. After 5 min, 2.0 mL of saturated sodium carbonate solution were added and the optical density of the solution at 640 nm was measured after one hour. The amount of phenolics (expressed as µg chlorogenic acid/g fresh weight) was calculated from a standard curve (20-100 µg chlorogenic acid/mL) prepared at the same time.

Degree of Browning

A subjective internal browning score, based on a 0-5 scale (0=none, 1=trace, 2=slight, 3=moderate, 4=severe, and 5=extremely severe) was developed for the final 28 week analysis during the first study year. A minimum of 30 apples were

cut in half and their internal browning assessed immediately.

In addition, the objective method of Mapson *et al.* (1963) for assessing the browning tendency of a cut surface was adapted for apples. Browning tendency was measured using a Hunterlab colorimeter Model D25L-3 and expressed by difference (ΔL) in Hunter 'L' value of the cut apple surface immediately after cut and after a 30 min period.

Polygalacturonase Extraction and Assay

Preparation of enzyme extracts was similar to the method of Jen and Robinson (1984). A 100 g sample of apple flesh was homogenized for one minute in 100 mL water, filtered through a milk filter, and the residue was resuspended in 100 mL 1.0M NaCl. The residue was further homogenized by a Polytron (Brinkmann) for one minute, and the suspension was adjusted to pH 6.0 and stirred slowly at 4°C for one hour, taking care to maintain the pH at 6.0. After one hour the suspension was filtered through a milk filter, the residue discarded and the supernatant was centrifuged at 27,000xg for 15 min. The resulting supernatant was desalted on a Sephadex G-25 column and used for assay of polygalacturonase activity.

The assay of polygalacturonase activity was based on the hydrolytic release of reducing groups from polygalacturonic acid, according to the method of Gross (1982). Fifty µL of enzyme extract were added to 200 µL of 0.2% of polygalacturonic acid (Sigma Chemical Co.) in 37.5 mM sodium acetate buffer, pH 4.4. The mixture was incubated for 20 h at 30°C, then terminated with 1.0 mL of cold 100 mM borate buffer (pH 9.0). Two hundred microliters of 1% 2-cyanoacetamide (Sigma Chemical Co.) were added to the reaction mixture, stirred and immersed in a boiling water bath for 15 min. After equilibration to 25°C, the absorbance at 276 nm was measured using a Varian Cary 219 spectrophotometer. One unit of PG activity was defined as the quantity of enzyme capable of catalyzing the formation of 1 nmoles of reducing sugar per minute under assay conditions.

Electrolyte Leakage

The method of Furmanski and Buescher (1979) for measurement of electrolyte leakage and internal conductivity was utilized to assess membrane permeability. Mescocarp tissue samples were prepared by slicing cylinders cut with a no. 8 cork borer into discs (2 mm thick and 12 mm in diameter), washing 3 times with distilled water and incubating in 0.4M mannitol for 30 min at 25°C with intermittent shaking. Electrolyte leakage was determined with a conductivity meter (Chemtrix Type 700, Hillsboro, OR) after incubating the discs and again after autoclaving (121°C, 15 p.s.i. for 30 min) and cooling to 25°C (for total electrolyte measurement). Electrolyte leakage after 30 min was calculated as percent of total electrolytes.

Firmness

A McCormick hand operated puncture tester was used to measure the firmness of each apple. The tester had a 25 lb spring and a 11.1 mm (7/16 in.) diameter punch. The plunger was placed against the sample and steadily increasing force was applied until the plunger penetrated to the inscribed line (Bourne 1982). Readings were taken on two pared areas on opposite sides of each fruit, midway between the stem and the blossom ends. At least 12 apples were sampled from each of the three tree replicates. Apples were taken out of storage or holding and allowed to equilibrate to room temperature (~25°C) for 8 prior to measurement.

RESULTS AND DISCUSSION

Similar trends were observed in all parameters during both years; therefore, only the data from the 1988/89 season with be presented. In both years there was a reduction in total PPO activity with storage time under both storage conditions (Barrett *et al.* 1991). Activity was slightly lower in apples stored under high CO_2 conditions than in normal CA stored apples, and those held an additional 7 days at 18°C had exhibited a sharp reduction in total activity by 7 weeks of storage (Barrett *et al.* 1991).

The concentration of total phenols was fairly stable, and ranged between 700-1000 µg/g under both storage conditions throughout the entire storage season (Fig. 1). There were no significant differences in phenol content between apples analyzed immediately after harvest and those held in air. CoSeteng and Lee (1987) found that the total phenol concentration in 'Classic Delicious' apples was relatively constant throughout the storage period. The total phenolics concentrations of seven apple varieties tested by these authors ranged from 1500-2000 µg/100 g and compared well with those obtained in the present study. Most

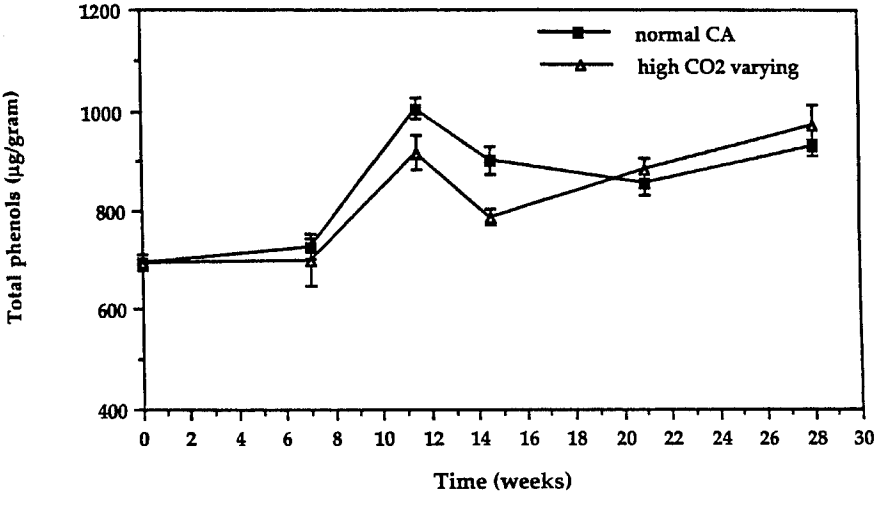

FIG. 1. TOTAL PHENOLS IN STORED 'DELICIOUS' APPLES

previous studies have found that total phenol concentrations remain fairly constant or decrease slightly during cold storage (Harel *et al.* 1966; Ingle and Hyde 1968; Vamos-Vigyazo *et al.* 1985b), but few studies have examined phenolic changes following removal from CA storage. Siriphanich and Kader (1985) found that lettuce stored under high CO_2 conditions did not exhibit much change in phenol concentration until the lettuce was transferred to air, at which time phenolics and browning increased. In light of these results, it appears that the correlation of phenolics to browning is weak.

Internal browning scores (data not shown) of apples analyzed immediately after removal from CA storage for 28 weeks were zero for normal CA stored apples and 1.2±0.16 for apples stored under varying high CO_2 conditions. Apples held 7 additional days at 18°C had internal browning scores of 0.6±0.16 after storage for 28 weeks under normal CA conditions, and 1.7±0.21 after storage under high CO_2 conditions. Those fruit stored under varying high CO_2 conditions were observed to be both browner initially and to darken faster after 30 min in air than normal CA stored apples. Liu and Pan (1989) also found that 'Delicious' apples stored under varying high CO_2 conditions similar to those used in this study had significantly greater internal flesh browning than normal CA stored apples after 6 months, and browning increased dramatically during a 7 day holding period.

In general, the browning tendency, or delta 'L' value, declined during storage (Fig. 2). Apples stored under normal CA and varying high CO_2 conditions exhibited similar trends and were not significantly different in delta 'L' value, although varying high CO_2 stored apples showed a slightly up and down pattern. This may be explained by the fact that O_2 concentrations increased, then decreased in this storage treatment and most probably affected browning tendency. Holding apples in air for an additional 7 days at 18°C (data not shown) resulted in a similar

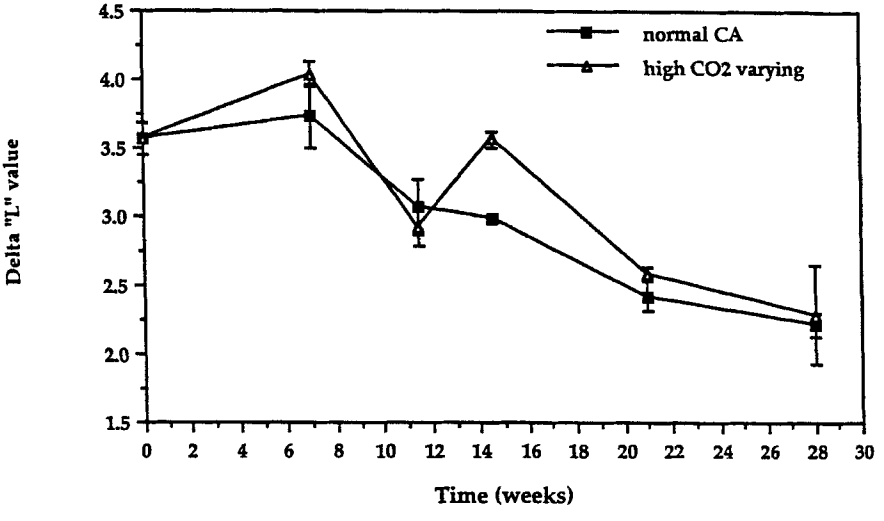

FIG. 2. BROWNING TENDENCY IN 30 MIN. IN STORED 'DELICIOUS ' APPLES

downward trend in delta 'L' value, and there were no significant differences between storage treatments, nor did values differ much from the browning tendency observed in immediately analyzed apples.

Polygalacturonase (PG) activity in the cell wall fraction at harvest in the first year of storage was measured to be 0.0673 (±0.0007) units/gram-minute and 0.0604 (±0.0007) units/gram-minute in the second year. Although all samples analyzed exhibited PG activity in the cell wall fraction at harvest, there was no measurable activity at any time during the storage period. Either PG activity decreased during storage to the point where it was not detectable with the method used, or its activity was inhibited. The analytical method, which was sensitive to nmole concentrations of reducing carbohydrate, had not been used previously for apples. Bartley (1978) used a viscometric assay to identify PG activity in 'Cox's Orange Pippin' apples stored in air at 3.3°C for 5-8 months and reported a PG activity of 0.36 µmol/g/h. The activities of enzymes such as pectin esterase, ß-galactosidase, endo-ß-1,4-glucanase and PG are all quite low in ripening apples, and the processes controlling apple softening remain unresolved.

Percent change in electrical conductivity increased from the harvest value of 24.7% to an apparent maximum of 33% at 11.5 weeks under both storage conditions (Fig. 3), then decreased throughout the remainder of the storage season. There was no significant difference between the normal CA and high CO_2 stored apples at any time period. Lewis and Martin (1969) also noted an upward trend in leakage from excised 'Jonathan' (stored 160 days) and 'Sturmer' (stored 80 days) apple discs during the first three-fourths of the storage period, followed by a significant downward trend until the end of storage. Apples held an additional 7 days in air at 18°C (data not shown) did not exhibit the same tendencies as those analyzed immediately. Although leakage was slightly higher at 11.5 weeks in normal CA stored apples and at 14 weeks in varying high CO_2 stored apples, the values were fairly constant (~27%) throughout storage. Furmanski and Buescher

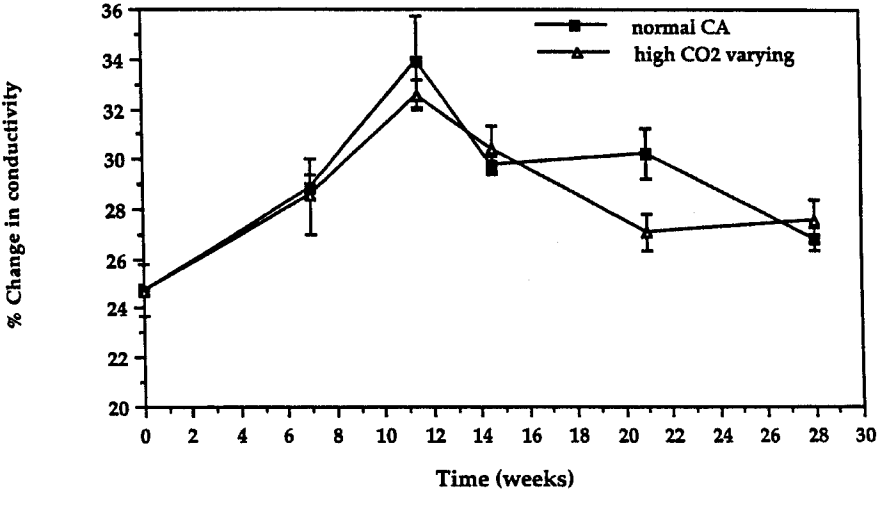

FIG. 3. ELECTROLYTE LEAKAGE IN STORED 'DELICIOUS' APPLES

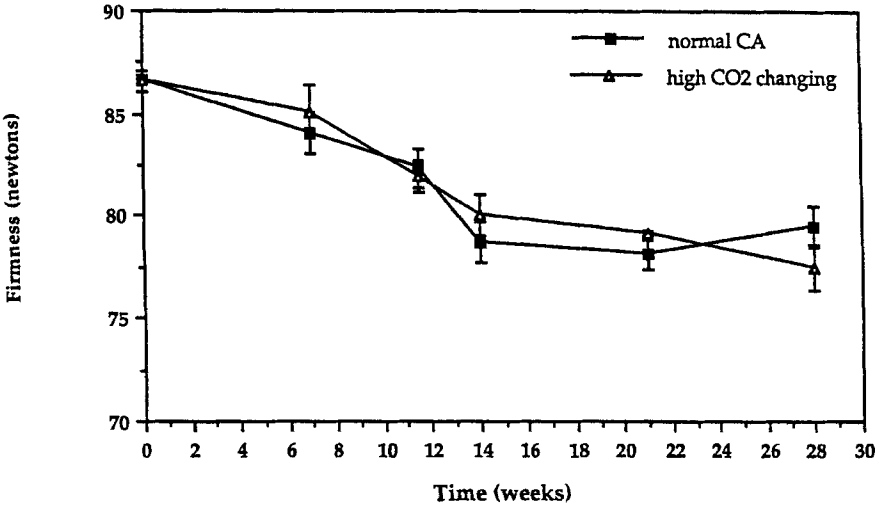

FIG. 4. FIRMNESS OF STORED 'DELICIOUS' APPLES

(1979) noted that electrolyte leakage increased, then decreased, in peaches which undergo chilling injury. The authors suggested that the reduced leakage observed after chilling temperatures were terminated was due to cation binding and therefore inability to conduct electrical current.

There was a general decline in the firmness of apples in both years of the storage study (Fig. 4). Firmness values declined from 87.8 N to 79.7 N after 28 weeks of storage. There was no significant difference between the storage environments utilized in either year at any storage time. Couey and Olson (1977) applied 10-20% CO_2 treatments to 'Golden Delicious' apples after harvest and found that, while there was no effect immediately after treatment, after 7 days at 18°C the apples exposed to elevated CO_2 levels had softened less than untreated apples. Apples which were held in air an additional 7 days (data not shown) showed accelerated reductions in firmness with storage time and by 14 weeks of storage, firmness values for both normal CA and high CO_2 stored apples had stabilized at approximately 63 N. Results from the present study do not indicate improved firmness as a result of long term high CO_2 treatments, and in some cases shown increased browning. Liu and Pan (1989) noted similar results in 'Delicious' apples stored under varying high CO_2 conditions for 6 months. The authors concluded that although it may be possible to substitute rapid cooling procedures with high CO_2 atmospheres in the early period of CA storage in order to save on cooling costs, these treatments offer no improvements in firmness.

In conclusion, there was no significant difference in total phenolics, browning tendency, electrolyte leakage or firmness between 'Delicious' apples stored under elevated, varying CO_2 conditions and normal CA stored apples. Therefore, there appears to be no advantage to using the storage regime described as a means of reducing apple browning or softening. While other investigators have examined

the effects of CA storage on these parameters, none have used the regime described in this paper, nor have they examined differences in these parameters following a simulated marketing period.

After approximately 7-14 weeks in CA storage, 'Delicious' apples showed significant losses in PPO activity, browning tendency and firmness. Electrolyte leakage reached a maximum at 11.5 weeks and declined, while total phenolics remained fairly constant. The changes observed may indicate a loss in membrane integrity and decompartmentalization after 7-14 weeks of storage. This decomposition would potentially facilitate enzymatic browning in apples during CA storage. Polygalacturonase activity was only detectable at harvest, however, which may indicate that the role of PG in apple softening is not as significant as once thought. Holding apples for additional time in air following removal from CA storage appears to accelerate changes in these characteristics.

Future research efforts might be directed at better defining the effect of O_2 and CO_2 on general cell integrity and the enzymatic browning reaction in particular and on improvements in PG determination.

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