Phenolic Compounds and Their Changes in Apples during Maturation and Cold Storage

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Phenolic compounds have been determined by HPLC in the flesh and skin of three apple cultivars (Golden Delicious, Empire, Rhode Island Greening) during maturation and cold storage. The main phenolic compounds in all three apple cultivars were found to be epicatechin and procyanidin B_2, rather than chlorogenic acid which previously had been reported as the major phenolic compound in apples. The concentration of individual phenolics in apple flesh decreased sharply during the early stage of development and then remained relatively constant during maturation and storage. There was a direct correlation between concentration of polyphenols in the flesh and in the skins. The tendency to brown decreased throughout the fruit development and maturation period.

The enzymatic browning of fresh and processed fruits is a major problem for the industry. Discoloration of apple tissue is mainly due to oxidation of phenolic compounds by polyphenoloxidase (PPO). The rate of the enzymatic browning in apples depends on the PPO activity and polyphenol concentration (Harel et al., 1964; CoSeteng and Lee, 1987). Some studies have shown that chlorgenic acid is the major phenolic compound in apples and that it decreases rapidly during the early stage of fruit development to reach an almost steady level at maturity (Walker, 1963; Vamos-Vigyazo et al., 1976). The total phenol concentration in apples is reported to stay at a relatively constant level during storage (Harel et al., 1966; CoSeteng and Lee, 1987). However, some individual phenolic compounds, such as catechins (Mosel and Herman, 1974; Kolesnik et al., 1977) and chlorogenic acid (Walker, 1962; CoSeteng and Lee, 1987), have been reported to change in concentration during storage.

Since individual phenolic compounds have been shown to vary in their browning rates (Oleszek et al., 1989), it is important to know the concentration of individual phenolics in apples and their changes during maturation and storage. This was the purpose of this study.

MATERIALS AND METHODS

Apples. Three apple cultivars, Golden Delicious, Empire, and RI Greening, grown during the 1987 season at our Experiment Station orchard were used for this study. Samples consisting of 10–12 apples for each experiment were obtained from the designated three trees every 2 weeks from June 24 to the harvest time in September. The samples were examined and analyzed immediately after picking. For the storage study, apples harvested at commercial maturity in September were collected in 25-kg crates and stored in normal cold-storage conditions at 0 °C (95% RH) for Golden Delicious and Empire apples and 2 °C (95% RH) for RI Greening apples for over 6 months. Sampling was carried out once a month until April 13, 1988.

Analysis of Phenolic Compounds. Five apples of each cultivar were peeled by hand, and 20 g of skin and 50 g of flesh were placed in beakers with 200 mL of methanol and 1 g of ascorbic acid. The samples were homogenized in a blender, filtered, and evaporated under vacuum. Extraction and fractionation into acidic and neutral phenolics using C_{18} SEP-PAK cartridges (Waters Associates) and HPLC analysis of individual phenolics were followed by the method of Jaworski and Lee (1987). Individual compounds were identified by comparison of their retention time and UV spectra with those of the standards as reported previously (Jaworski and Lee, 1987; Oleszek et al., 1988). Duplicate analyses were conducted on duplicate samples. The concentration of phenolics was expressed as micrograms per gram of fresh weight.

Measurement of Browning. Degree of browning tendency was measured on a Hunterlab colorimeter, Model D25L-3. A slice of apple (5-cm diameter) was removed from the midsection of the fruit, and the exposed flesh side was mounted on a light source of the instrument. Degree of browning tendency was expressed by the difference in Hunter L values of the fruit immediately after being cut and after being held 7 min.

RESULTS AND DISCUSSION

Since chlorogenic acid and procyanidin B_2 eluted together as one peak from the C_{18} column, the fractionation using pH-adjusted C18 SEP-PAK cartridges was necessary to separate the compounds before HPLC analysis. Chlorogenic acid that passed through the acidic cartridge was collected and measured separately by HPLC, while procyanidin B_2 that adsorbed on the cartridge with the remaining neutral fraction was removed with methanol and then analyzed by HPLC (Jaworski and Lee, 1987). Many previous reports have shown chlorogenic acid to be the major phenolic compound in apples (Walker, 1963; Ingle and Hyde, 1968; Vamos-Vigyazo et al., 1976). However, none of these reports showed the isolation or measurement of procyanidin B_2. Our results showed that the major phenolics in both apple flesh and skin were epicatechin and procyanidin B_2. This confirms the previous report by CoSeteng and Lee (1987).

As shown in Table I, the phenolics in apple flesh consisted of five main compounds: epicatechin, procyanidin B_2, phloretin xylogalactoside, phloretin glucoside, and chlorogenic acid. The skins contained an additional five quercetin glycosides (glucoside, galactoside, xyloside, arabinoside, rhamnoside) as reported previously (Dick et al., 1987; Oleszek et al., 1988). It is interesting to note that the relative amount of phenolics in the skins was several times higher than that in the flesh and that quercetin glycosides were found only in the skins. This clearly indi-

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Table I. Concentration of Phenolic Compounds in Flesh and Skins of Apples during Maturation and Storage (from Aug 5, 1987, to April 13, 1988)

	concentration, μg/g fresh wt (av ± SD)									
cultivar/tissue	epicatechin	procyanidin B_2	phloretin xylogalact	phloretin gluco	chlorogenic acid	quercetin galact	quercetin gluco	quercetin xylo	quercetin arabino	quercetin rhamno
Golden Delicious										
flesh	40 ± 9	60 ± 19	20 ± 4	10 ± 3	40 ± 11	nd[a]	nd	nd	nd	nd
skin	210 ± 130	200 ± 132	130 ± 51	150 ± 40	40 ± 29	290 ± 84	70 ± 31	100 ± 32	130 ± 28	230 ± 71
Empire										
flesh	10 ± 11	40 ± 29	10 ± 3	10 ± 2	50 ± 31	nd	nd	nd	nd	nd
skin	130 ± 107	120 ± 87	60 ± 44	120 ± 36	30 ± 21	220 ± 86	110 ± 31	110 ± 32	140 ± 36	200 ± 66
Rhode Island Greening										
flesh	140 ± 22	150 ± 31	30 ± 6	10 ± 5	60 ± 19	nd	nd	nd	nd	nd
skin	670 ± 214	600 ± 210	230 ± 59	100 ± 29	60 ± 44	370 ± 96	130 ± 77	150 ± 57	200 ± 54	220 ± 37

[a] nd = not detected.

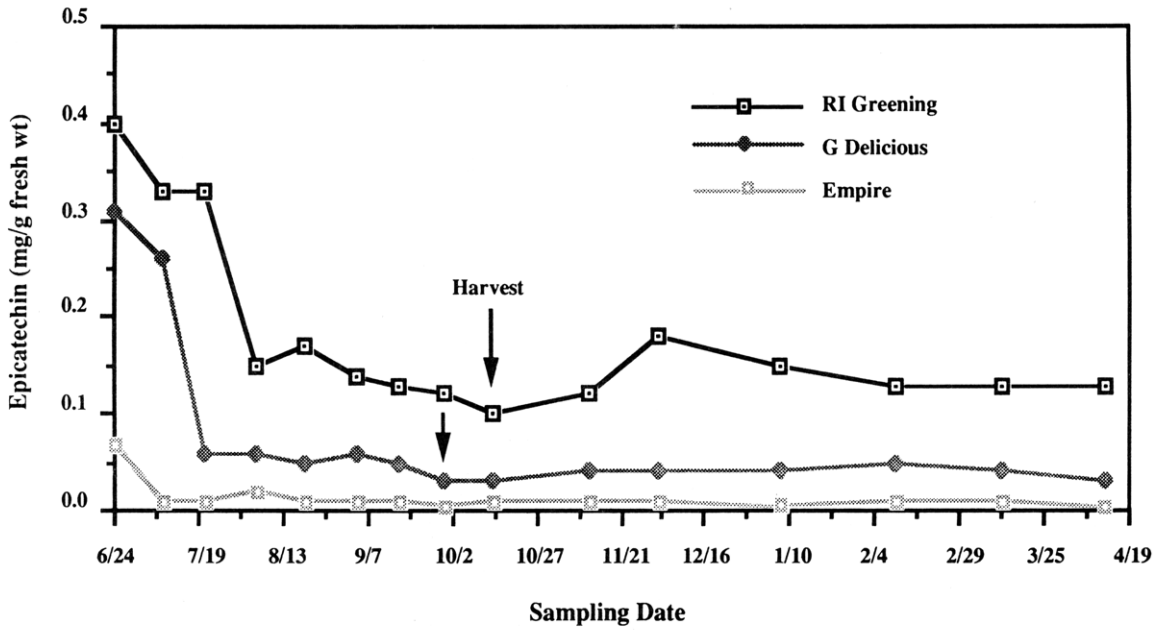

Figure 1. Epicatechin content in flesh of three apple cultivars during maturation and storage.

cates that the enzymatic browning of apples can be minimized in products if the skins were excluded in the processing.

A large cultivar variation in concentration of phenolics was also noticed. Rhode Island Greening apples contained higher concentrations of epicatechin, procyanidin B_2, and phloretin xylogalactoside in the skins and flesh while the Empire cultivar contained the lowest. The Empire apple cultivar was reported to be the lowest in phenolics and browning rate among seven apple cultivars (CoSeteng and Lee, 1987). The phenolic concentration of Golden Delicious apples was between that of RI Greening and Empire. Since concentrations of individual phenolics stayed at about the same level from Aug 5 to the end of the storage period, average concentrations are presented during these maturation and storage periods (Table I).

The influence of cultivar was compared during maturation and storage. Figure 1 shows the relative change of epicatechin in the flesh of the three cultivars during maturation and storage. All showed the same pattern of change. The epicatechin content was the highest during the fruit development stage and then rapidly decreased up to Aug 5, after which it stayed at approximately the same level during maturation and storage. RI Greening apples showed some increase and decrease during storage but maintained the same high level throughout maturation and storage compared to that in Golden Delicious and Empire apples. Other individual phenolic compounds in apple flesh showed changes similar to that of epicatechin during maturation and storage.

The concentration of phenolics in the skins also decreased rapidly during the fruit development stage (between June and July) and then stabilized throughout the maturation and storage period. Figure 2 shows a typical change in epicatechin in RI Greening apple flesh and skins during fruit development, maturation, and storage. Epicatechin in the skins showed a peak on July 8, 1987, decreased drastically during the following 2 weeks, and then maintained a stable level thereafter. Other phenolics in the skin appeared to have reached a maximum before June 24; therefore, they showed only the rapid phase of decrease during the same period. All three cultivars showed a similar pattern of changes in the apple skins.

A decrease in phenolics during fruit development appeared to be the general trend of changes as reported previously (Harel et al., 1966; Mosel and Herman, 1974; CoSeteng and Lee, 1987). However, there is some disagreement concerning changes in phenolics during maturation and storage. Mosel and Herman (1974) and Kolesnik et al. (1977) reported that the concentration of catechins in apples had a tendency to decrease for several months during storage. Our results showed that the concentration of the major phenolics, epicatechin, procyanidin B_2, and phloretin glycosides in apple flesh

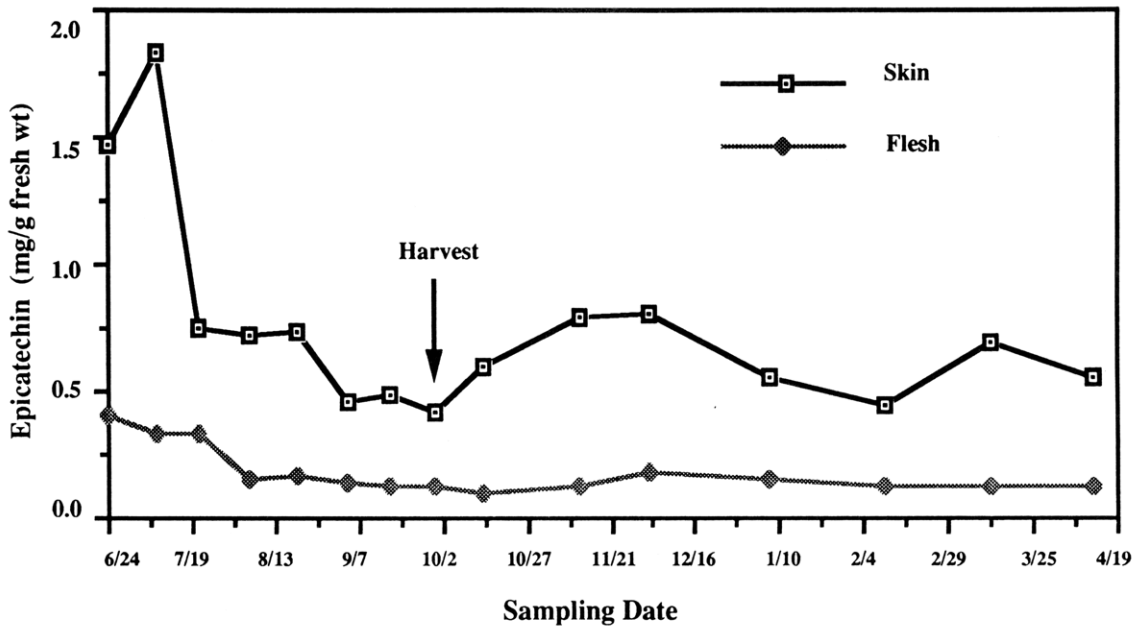

Figure 2. Epicatechin content in flesh and skins of Rhode Island Greening apples during maturation and storage.

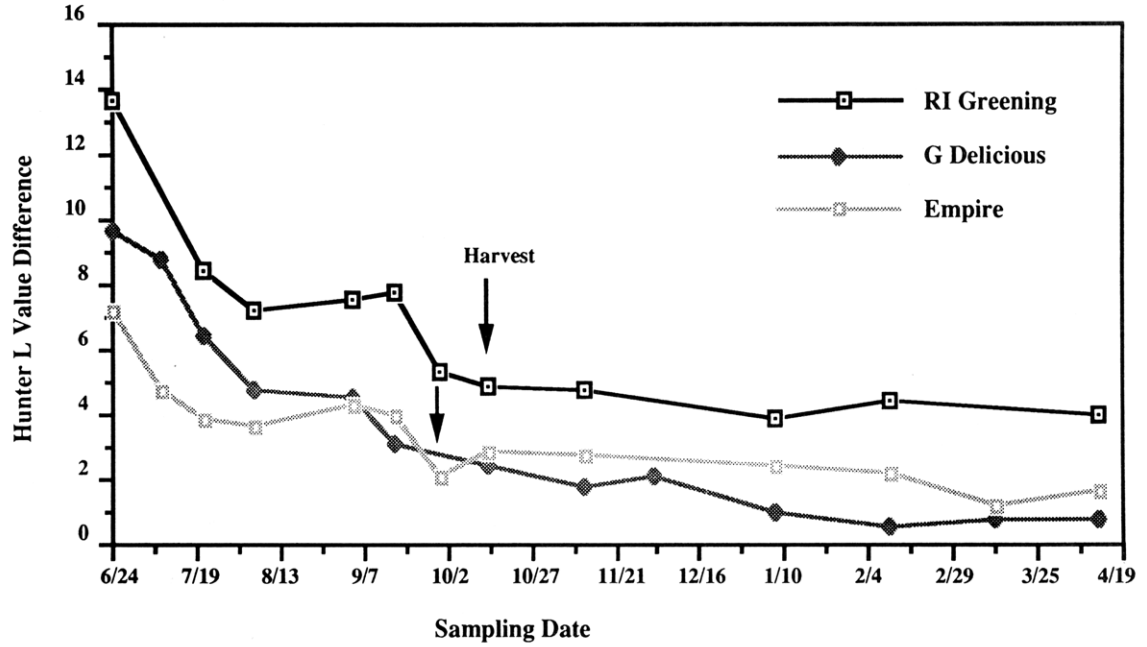

Figure 3. Browning tendency of apple flesh during maturation and storage.

remained at a relatively constant level from the maturation period, Aug 5, 1987 to the end of storage, April 18, 1988.

Figure 3 shows the browning tendency of apple flesh during maturation and storage as measured by Hunter L value. The Hunter L value difference decreased rapidly during June and July in the same pattern as was observed in phenolics. However, slow but steady and continuous decreases in the browning tendency were observed until the end of storage. RI Greening apples were the highest in browning tendency among the three cultivars throughout maturation and storage. The high browning tendency of RI Greening apples can be correlated with their high concentrations of phenolics. Hunter L value differences of Empire apples were slightly higher than those of Golden Delicious apples during storage, even though Empire apples contained less phenolics than Golden Delicious. Since enzymatic browning is dependent on several factors, it is difficult to explain our observation only on the basis of phenolics. However, it may be explained by the fact that the initial Hunter L value of Golden Delicious apples was lower due to the internal browning during storage; therefore, there was not much capacity to show an additional large change during the browning tendency measurement.

It is concluded that epicatechin and procyanidin B_2 are the major phenolics in apples rather than chlorogenic acid as reported. Since epicatechin and procyanidin B_2 showed much higher rates of browning and higher intensity of color than that of chlorogenic acid (Oleszek et al., 1989), these two compounds appeared to be the major factor in the enzymatic browning of apples. Concentration of phenolics decreased rapidly during the months of June and July and then stabilized to a relatively low level and stayed at the same level during maturation and cold storage. The tendency of browning was closely related to the content of phenolics during maturation and storage.

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Cell Wall Bound and Soluble Peroxidases in Normal and Dwarf Tomato

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Normal and dwarf tomato shoots were analyzed for their peroxidase isoenzyme content and total peroxidase activity. Both plant types were shown by electrophoresis to contain three isoenzymes that moved toward the anode and one that moved toward the cathode. Both types contained a cell wall bound peroxidase that was electrophoretically different from the four soluble isoenzymes. Cell wall bound peroxidase was separated from soluble peroxidase isoenzymes in crude extracts by using purified cell walls. With this method, accurate measurements of the total cell wall bound and soluble peroxidase activities were obtained. Cell wall bound peroxidase accounted for 30% and 56% of the activity in crude extracts of normal and dwarf plants, respectively. Total peroxidase activity in the normal plant was 74% cell wall bound and 81% in the dwarf plant. Cell wall bound peroxidase activity was 5.2 times greater in the dwarf when compared to the normal plant.

The ionic binding of peroxidases to cell walls has been studied for quite some time, and as yet, no definitive agreement can be reached as to how much or what kind of peroxidases are, in fact, bound to the cell wall. A number of review articles have summarized these studies (Lamport, 1965; Fry, 1986; Cassab and Varner, 1988). It would appear that the amounts and types of peroxidases vary depending on the phylogeny and ontogeny of the plant, and the issue is further complicated by attempts to relate the results of tissue culture model systems back to the whole plant (Mader et al., 1975; van Huystee and Chibbar, 1987).

It has long been known that dwarf plants generally contain higher peroxidase activity than their normal counterparts (Kamerbeek, 1956; McCune and Galston, 1959). However, it is not known whether this dwarfing is the result of increased or premature lignification due to increased peroxidase activity or whether increased peroxidase activity is caused by the dwarfing due to other factors. Although the peroxidase activities in dwarf plants are greater than in the normal plants, it is not known whether this increased activity is due to the cell wall ionically bound peroxidases or the nonionically bound, soluble peroxidases, or both.

This present research describes the types and amounts of total nonionically bound, soluble peroxidases and the ionically bound cell wall peroxidase present in dwarf and normal tomato plants. It further defines the peroxidase that is associated with the cell wall and its total activity in relation to the other soluble, nonionically bound peroxidase isoenzymes.

MATERIALS AND METHODS

Plant Material. Normal (d^+) and dwarf (d^x) tomato plants (*Lycopersicon esculentum* Mill.) were grown in a greenhouse from seed to a height of ca. 1 and 0.4 m, respectively, and were ca. 6 months old. The slow growth rate and morphology of the dwarf plant were described by Rick and Butler (1956), and the