Enzymatic Browning in Relation to Phenolic Compounds and Polyphenoloxidase Activity among Various Peach Cultivars

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The major phenolic compounds and the polyphenoloxidase (PPO) activity in various peach cultivars grown in New York during the 1987 and 1988 seasons were studied to correlate them with the degree of browning. The concentration of individual phenolic compounds decreased steadily during maturation and remained low until harvest time. PPO activity followed a pattern similar to that of the phenolics during ripening. Large seasonal and cultivar variations in certain phenolic compounds and PPO activity were observed. The degree of browning closely correlated with the phenolic content and enzyme activity.

Phenolic compounds and polyphenoloxidase (PPO) are, in general, directly responsible for the enzymatic browning reactions in damaged fruits during postharvest handling and processing. The reactions produce undesirable changes in color, flavor, and nutritive value of the product. The degree of browning among different fruit cultivars is known to vary due to differences in phenolic content and PPO activity. Wide variations in total tannin content among different peach varieties have been reported (Blake and Davidson, 1941; Grice et al., 1952). Large differences in tannin content due to different growing seasons and growing areas have also been observed (Guadagni and Nimmo, 1953). Changes in PPO activity during the ripening of Elberta, Salvey, and Redhaven peaches have been documented (Craft, 1961; Harel et al., 1970; Flurkey and Jen, 1978), and the biochemical characteristics of the enzymes from Elberta and Redhaven peaches have been investigated (Reyes and Luh, 1960; Flurkey and Jen, 1978).

The relationship of the rate of browning to phenolic content and PPO activity has been reported for various fruits such as apples (CoSeteng and Lee, 1987) and grapes (Wisseman and Lee, 1980; Lee and Jaworski, 1988). Similar studies have not been conducted on the important peach cultivars. The purpose of this research was to analyze the individual phenolic compounds and the PPO activity in various peach cultivars grown in New York State and to correlate them with the degree of browning during maturation and at harvest. Hopefully, the data would lead to the selection of cultivars that would minimize browning during postharvest handling and processing of peaches.

MATERIALS AND METHODS

Peaches. All fruits were obtained from the New York State Agricultural Experiment Station orchards during the 1987 and 1988 seasons. Random sampling was carried out every 7–14 days from selected trees starting with the green immature stage (mid-July) to the mature stage (mid-September). Each sample, composed of 8–12 peaches, was brought to the laboratory immediately after harvest and then peeled and analyzed.

Analysis of Phenolic Compounds. Phenolic compounds were extracted with methanol, separated into acidic and neutral fractions with C_{18} SEP-PAKs (Waters Associates), and then analyzed for individual phenolic compounds by HPLC (Jaworski and Lee, 1987).

Identification of Chlorogenic, Neochlorogenic, and Caffeic Acids. Phenolic compounds were extracted with methanol from about 3 kg of fruit and then separated into acidic and neutral fractions on a C_{18} column (55–105 μm, 3 cm × 26.5 cm) (Jaworski and Lee, 1987). The acidic fraction was applied to a Sephadex LH-20 column (1.5 cm × 36 cm), which gave three peaks after elution with 2% acetic acid solution. The three individual acidic phenolics were concentrated and subjected to identification by HPLC, TLC, spectrophotometry, and hydrolysis (Oleszek et al., 1988). The EI mass spectra were measured with a Finnigan 3300 quadrupole mass spectrometer. The samples were introduced into the ion source with a direct probe and were ionized with 70-eV electrons. Identification of the compounds was made by comparing their spectra with those of standards.

Measurement of PPO. Crude PPO was obtained by extracting peaches with McIlvain's buffer at pH 6.5. The activity was measured by using catechol as the substrate (Wissemann and Lee, 1980). One unit of PPO activity was defined as an increase of 0.001 unit of absorbency/min per g of peach at 420 nm.

Measurement of Browning. The peeled peaches were ground in a Waring blender with an equal weight of distilled water for 1 min, immediately poured into a glass cylinder (7.5-cm diameter × 6-cm height), and then the L value was measured with a Hunter color meter (Model 25D). The degree of browning was expressed as the L value differences at times 0 and 60 min. All analyses were carried out in duplicate.

RESULTS AND DISCUSSION

The major phenolic compounds found in peaches were catechin, procyanidin B3, chlorogenic acid, neochlorogenic acid, and caffeic acid. The identity of catechin was confirmed by the method of Oleszek et al. (1988). Procyanidin B3 was tentatively identified according to its spectrum and its retention time on the HPLC column. Confirmation of chlorogenic acid, neochlorogenic acid, and caffeic acid was made by comparing the retention time of HPLC, hydrolysis products, spectrophotometric spectra, and EI mass spectra with those of standards. The EI spectra of chlorogenic acid were observed $(M)^+$ at m/z 354 (0.5%), $(M - H_2O)^+$ at m/z 336 (2.0%), $(\text{caffeic acid})^+$ at m/z 180 (45.7%), $(\text{caffeoyl})^+$ at m/z 163 (100%), and $(\text{caffeoyl} - H)^+$ at m/z 162 (42.2%). The spectra of neochlorogenic acid were observed $(M - H_2O)^+$ at m/z 336 (3.5%), $(\text{caffeic acid})^+$ at m/z 180 (31.0%), $(\text{caffeoyl})^+$ at m/z 163 (100%), and $(\text{caffeoyl} - H)^+$ at m/z 162 (22.6%). These spectral data were very similar to those reported previously for chlorogenic acid and neochlorogenic acid (Sakushima et al., 1985).

Figure 1 shows a general pattern of change in phenolics during maturation of Madison peaches. The con-

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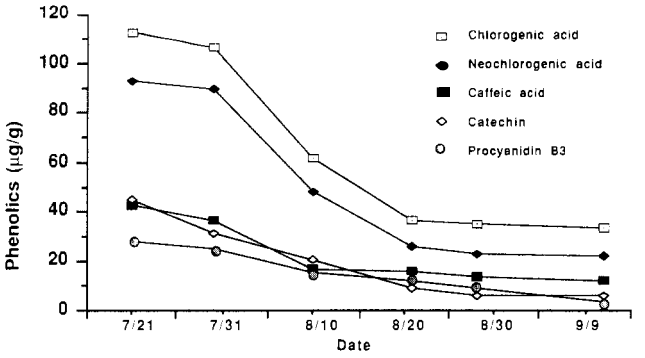

Figure 1. Phenolic content of Madison peaches during ripening (1987 crop).

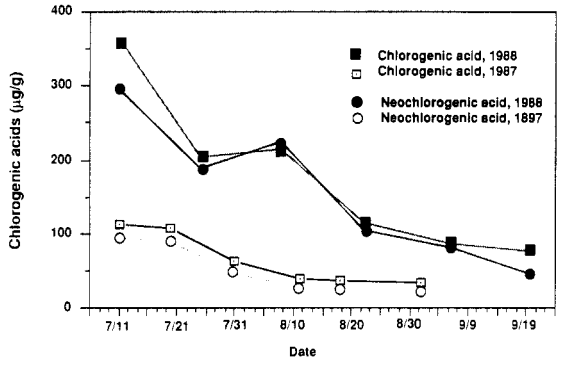

Figure 2. Chlorogenic acids in Madison peaches grown in 1987 and 1988.

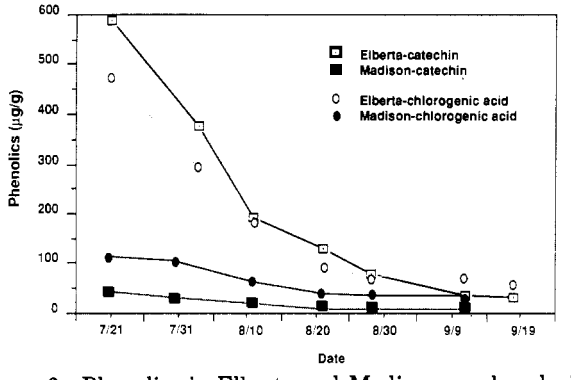

Figure 3. Phenolics in Elberta and Madison peaches during maturation (1987 crop).

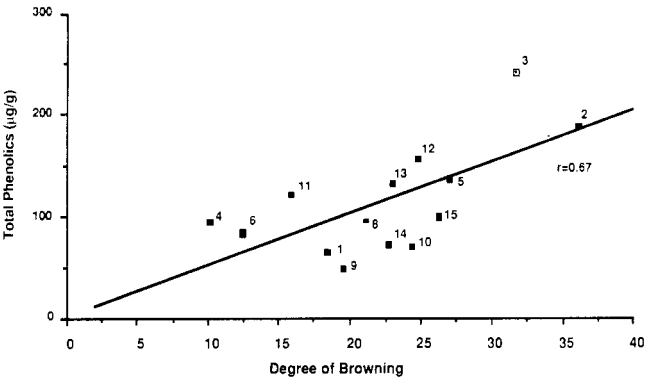

Figure 4. Relationship between browning (measured by Hunter L value) and total phenolics among different peach cultivars (numbers refer to cultivars in Table I).

tent of all five phenolic compounds decreased during ripening and stayed at the lowest level for 2 weeks before harvest. All other peach cultivars followed similar patterns of change during maturation in 1987 and 1988. The content of chlorogenic acid and neochlorogenic acid was high throughout the maturation period.

There was a significant difference in the concentration of certain individual phenolic compounds in the same peach cultivar between the two growing seasons. In 1988, the content of chlorogenic acid and neochlorogenic acid of Madison peaches during the ripening period was significantly higher than that of the same cultivar grown in 1987 (Figure 2). Catechin, procyanidin B3, and caffeic acid, on the other hand, showed similar levels in peaches grown in 1987 and 1988 (data not shown).

A large difference in concentration of certain phenolics was observed among different peach cultivars: The content of chlorogenic acid, neochlorogenic acid, and catechin in Elberta was several times higher than that of Madison at the early stage of ripening. This difference became smaller toward harvest time but still was significant (59 vs 25 μg/g for neochlorogenic acid and 35 vs 6 μg/g for catechin) as shown in Figure 3.

The cultivar differences in the content of individual phenolics during maturation reflected the large cultivar variation that was observed at harvest (Table I). Among 15 peach cultivars, Elberta peaches contained the highest amount of total phenolics at harvest followed by Eden and Reliance. Newhaven was the lowest in the total phenolic content, less than one-fifth that of Elberta. Overall, chlorogenic acid was the major phenolic compound in most of the peach cultivars. Relatively high concen-

Table I. PPO Activity, Phenolics, and the Degree of Browning in Various Peach Cultivars (1987 Crop)

cultivar	sol solids, %	PPO act., units/g	phenolics, μg/g					deg of browning, dL
			Chlrg	Neochlr	ProcB3	Catech	Caff	
1 Deep Early Hale	11.2	2635	7	9	27	15	6	18.4
2 Eden	12.8	4505	68	50	41	36	t	36.3
3 Elberta	13.0	2798	59	59	85	35	12	31.8
4 Harmony	9.5	1793	14	15	30	25	10	10.2
5 Hayhaven	12.3	2146	30	18	41	29	10	27.0
6 Harken	13.3	2105	28	13	27	17	5	12.5
7 La Red	11.4	2132	na[a]	na	na	na	na	9.1
8 Madison	11.6	1923	38	25	9	6	12	21.2
9 Newhaven	11.8	1722	14	17	4	7	6	19.6
10 Pekin	11.4	2390	17	16	24	13	t[b]	24.4
11 Redhaven	10.8	2299	41	33	28	20	t	15.9
12 Reliance	11.0	2361	74	54	18	11	t	24.8
13 Triogem	11.4	3340	42	24	40	16	10	23.1
14 Veecling	10.8	1449	23	20	11	7	11	22.8
15 Velvet	12.6	3196	25	21	27	12	6	26.3

[a] Not analyzed. [b] Trace amount.

trations of neochlorogenic acid, procyanidin B3, and catechin were observed in most cultivars while the caffeic acid content was relatively small. Since the contribution of chlorogenic acids to the enzymatic browning reaction was less significant than that of other phenolic compounds (Lee and Jaworski, 1988), attention should be given to catechin and procyanidin B3.

PPO activity in peaches also varied greatly among different cultivars (Table I). Eden showed the highest PPO activity (4505 units/g) among 15 cultivars, followed by Triogem and Velvet, which exceeded 3000 units/g. Veecling had the lowest in PPO activity (1449 units/g), which was less than one-third that of Eden. It was observed that the degree of actual browning of individual peach cultivars was correlated to its PPO activity: peach cultivars having higher PPO activity showed a higher rate of browning (e.g., Eden). Conversely, peaches low in PPO activity, such as Harmony, showed a lower rate of browning. When degree of browning was plotted against total phenolics (Figure 4), they showed a relatively close correlation ($r = 0.67$). A similar relationship was observed between degree of browning and the PPO activity ($r = 0.65$).

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Registry No. PPO, 9002-10-2; catechin, 120-80-9; procyanidin B3, 23567-23-9; chlorogenic acid, 327-97-9; neochlorogenic acid, 906-33-2; caffeic acid, 331-39-5.

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Sugar Content of Almond, Pecan, and Macadamia Nuts

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The individual sugar contents of five almond cultivars, pecan, and macadamia nuts were determined by a gas chromatographic method. Good separations of carbohydrates were obtained within a short time with use of a combination of OV-1 and OV-225 as stationary phase. Sucrose was identified as the main constituent in almond, pecan, and macadamia nuts. Small quantities of other sugars and sugar alcohols were detected.

Saura-Calixto et al. (1981) quoted reports from literature on determinations of sucrose in almond milk by means of paper chromatography; glucose and fructose in almonds; and fructose, glucose, sucrose, sorbitol, and inositol in almond shells by gas chromatography. Sucrose was reported to be the main constituent of carbohydrates in almonds. They confirmed these reports by determining total sugars by means of paper chromatographic, colorimetric, volumetric, and gravimetric methods and reported a mean value of 5.52 g of sucrose/100 g of almonds. They confirmed that sucrose was the main carbohydrate constituent. Saura-Calixto et al. (1984) again confirmed the presence of sucrose in almonds but also found traces of fructose, glucose, sorbitol, and inositol.

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