

Physicochemical Characteristics of Various Peach Cultivars

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Abstract

Physical and chemical characteristics of four white- and one yellow-fleshed peach cultivars were compared at optimum maturity stage. In addition, differences of phenolic composition, polyphenol oxidase (PPO) activities and PPO isozyme patterns in two peach types were also investigated. There are no significant differences in firmness and color values between two peach types, except for "Yumyung", a white-fleshed peach with the highest firmness, and "Hwangdo", a yellow-fleshed peach with the highest yellowness (b^*) value. In general, the soluble solid/titratable acidity ratios, ascorbic acid and total phenolic contents were significantly higher for white-fleshed peaches than those for yellow-fleshed peach ($p < 0.05$). Three major phenolic compounds, catechin, neochlorogenic acid and chlorogenic acid, were found in both the white- and yellow-fleshed peaches. Among them, catechin was the predominant phenolic compound in the white-fleshed peaches, followed by neochlorogenic acid, and chlorogenic acid. Meanwhile, neochlorogenic acid was present in the highest level of the yellow-fleshed peach, but levels of two other phenolic compounds were lower. PPO activities of the white-fleshed peaches were generally higher than that of the yellow-fleshed peach, with the exception of "Yumyung". The partially purified PPO of two peach cultivars have together three active bands including one major band and two minor bands, and there were no big differences in PPO isozyme patterns between two different peach cultivars.

Key words: white- and yellow-fleshed peaches, physicochemical characteristics, phenolic compounds, polyphenol oxidase (PPO), electrophoresis

INTRODUCTION

Peaches (*Prunus persica* Batsch) are a good source of acid, sugar, amino acid and vitamin A, which are important sources of functional beverages. In particular, demand for the peach fruits has increased in recent years because of its dessert quality and delicate flavor (1).

The production of peaches in Korea increased from 114,500 metric tons in 1990 to 147,000 metric tons by 1997 (2). In 1999, total peach production in Korea was 157,000 metric tons making it the sixth most important fruit behind apples, oranges, grapes, pears, and persimmons (2). The peach grows in many areas in Korea, and the leading areas are mostly Chungdo, Kyungsan, Youngcheon, Okcheon, Eumsung, and Youngduk (3).

Peach cultivars are broadly classified as white- and yellow-fleshed peaches, with the former accounting for about 80% of total production in Korea (2). Most of white-fleshed peach cultivars containing high amounts of sugars, flavors and fruit juice are presently utilized as fresh fruits, while the yellow-fleshed peaches with low sugars and high organic acids do as processed juice, nectarine, and sauce, etc. (1,2,4). Many extensive studies on the physical, chemical and sensory properties and volatile constituents of several peach cultivars have been undertaken (4-8). These quality characteristics of peaches were found to be affected by maturation, cultivar and processing (7,8). In addition, it was reported that there were con-

siderable differences in fermentation degree and processing characteristics according to peach cultivars (9-11). Therefore, it is very important to investigate the physical and chemical properties of peach cultivars prior to developing new processed peach foods.

Peaches are easily susceptible to undesirable enzymatic browning during postharvest storage and processing, which is catalyzed by polyphenol oxidase (PPO) and phenolic compounds (12). Wide variations in total phenolic content and PPO activity have been reported among various peach cultivars which were harvested in different growing seasons and areas (13-17). In general, the white-fleshed peaches are known to have greater polyphenol content and PPO activity than yellow-fleshed peaches (15,16). However, newly breeding white-fleshed peach cultivars high in phenolic compounds and low in PPO activity have recently been developed because phenolic compounds in peaches are receiving attention as potential sources of dietary antioxidants (17). In addition, few studies on the isolation and biochemical properties of PPO isozymes of peaches are available.

Recently, our research has been initiated to evaluate the physicochemical characteristics of white- and yellow-fleshed peaches grown in Youngduk, which produced 4,307 and 3,523 tons in 2000, respectively. The importance of these quality characteristics is especially pertinent in assessing for the manufacture of high quality of peach processed products, such as puree, sabeté, peach wine, vinegar, and dried fruit.

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The objective of our study was to compare some physicochemical characteristics of white- and yellow-fleshed peach cultivars, and to investigate further the phenolic composition and polyphenol oxidase (PPO) isozyme.

MATERIALS AND METHODS

Chemicals

Chlorogenic acid, catechin, gallic acid, Folin-Ciocalteu phenol reagent, and all electrophoretic reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate, polyethylene glycol and trifluoroacetic acid (TFA) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Dialysis membrane (MW 12,000) was purchased from Viskase Co. (Chicago, IL, USA). Centrifuge filter unit (MW 12,000) was obtained from Nalgene Bio. Tech. Acetone, acetonitrile and other solvents used in this study were of HPLC grade.

Materials

Four white peach cultivars (Changbang, Daegubo, Yumyung and Keedo), and one yellow cultivar (Hwangdo 2) grown in the field of Youngduk, Kyungbuk prefecture, Korea were harvested at optimum maturity on the basis of skin color from July 15 through September 10, 2000, and transported under refrigeration to the laboratory. The peach fruit was washed with tap water, air-dried and then peeled with a sharp knife. Physical (firmness, color) measurements and chemical (soluble solids, total acidity, L-ascorbic acid, total phenolics, polyphenol oxidase activity, and polyphenolic compositions) analysis of each peach flesh were determined in duplicate. The clingstone and non-clingstone peach cultivars could be differentiated unambiguously by visual observation after the peach was cut with a stainless steel knife.

Physical measurements

Peach fruits were randomly selected from each cultivar for color analysis. L^* (lightness), a^* (redness) and b^* (yellowness) values were measured using a portable colorimeter (Minolta Camera Co., Osaka, Japan). Replicate measurements for each slice were averaged, and the four slice values were averaged for each peach.

Firmness was determined on opposite pared cheeks of each fruit using a Rheometer (RE-3305, Yamaden Co., Japan) fitted with an 5-mm tip. The mean of the two measurements was expressed in newtons (N).

Sugar and total acidity

The soluble solids ($^{\circ}$ Brix) of expressed and filtered peach juice were determined with an Abbe refractometer (Atago N1, Atago Co., Japan). Titratable acidity of peach juices (5 mL) was titrated with 0.1 N NaOH, and then its contents were expressed as percentage (%) in terms of malic acid equivalent.

Ascorbic acid analysis

Ascorbic acid extraction was carried out according to the method of Wimalasiri and Wills (18). Fresh peach (10 g) was

homogenized in 80 mL of 3% citric acid, filtered, and then filled up to 100 mL with the same solvent. The extract (5 mL) was passed through Sep-Pak C_{18} cartridges to remove interfering compounds prior to HPLC analysis. The first 4 mL was discarded and next 1 mL was used for analysis. HPLC analysis was performed using a μ Bondapak-NH₂ column (3.6 mm \times 30 cm, Waters Associates, Milford, MA, USA) with a mobile phase of CH₃CN/H₂O (70:30) with 0.01 M ammonium dihydrogen phosphate (pH 4.3) at a flow rate of 1 mL/min with monitoring at 254 nm. Recovery rate for ascorbic acid was 99%. Duplicate analyses were conducted on duplicate samples.

Total phenolic content

The total phenolic contents of flesh peach samples were determined with the Folin-Ciocalteu reagent (19). Each peach sample (10 g) was homogenized twice with 80% aqueous methanol solution in a Waring blender for 2 min, filtered and then filled up 100 mL with 80% methanol. A sample solution (5 mL) was taken in a test tube and then Folin-Ciocalteu reagent (5 mL) was added. The solution was mixed thoroughly for 3 min, and then 5.0 mL of saturated Na₂CO₃ solution was added. The mixture was allowed to stand for 1 h with intermittent mixing. The blue color developed was measured on an Sinco UV-visible spectrophotometer (S-1100, Seoul, Korea). Total phenolic content of the peach sample was determined by comparison with the optical density values of different concentrations of a standard gallic acid, and the values for total phenolic contents were expressed in terms of gallic acid equivalent (GAE).

Analyses of phenolic compound compositions

Each peeled peach fruit (10 g) was homogenized twice in a blender (Cell master CM-100, Iuchi, Japan) with 50 mL of 80% aqueous methanol containing 0.02% K₂S₂O₅, filtered, and evaporated under vacuum. The sample was solubilized in 5 mL of 80% MeOH, and then passed through 0.25 μ m membrane filter (Nalgene). The phenolic composition of peach extracts was analyzed by HPLC according to the method of Chang et al. (17), with a slight modification. The sample was gradually eluted with two solvent systems; solvent A, 0.1% TFA in 5% MeOH; solvent B, 80% MeOH. Individual compounds were identified by comparison of their retention time and UV spectra with those of the standard compounds. Duplicate analyses were conducted on duplicate samples. The concentration of phenolics were expressed as micrograms per gram of fresh weight.

Partial purification of peach PPO

Peach PPO was prepared by a slight modification of the procedure reported by Billaud et al. (20). Fresh peach (10 g) was homogenized with 100 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing 2% hydrated polyvinylpyrrolidone (PVPP) and 0.02% NaHSO₃ in a Waring blender for 2 min. The homogenate was filtered and then centrifuged at 12,000 \times g for 30 min at 4°C, and the supernatant was fil-

tered through a 10 μm nylon filter and used as the crude extract for the assay. The crude extract was brought to 80% $(\text{NH}_4)_2\text{SO}_4$ saturation with solid $(\text{NH}_4)_2\text{SO}_4$. The precipitate was separated by centrifugation at $12,000\times g$ for 30 min at 4°C . The precipitate was dissolved in a small amount of the buffer used for homogenization and dialyzed at 4°C in the redistilled water for 24 hr. The sample solution in the dialysis tube was concentrated with polyethyleneglycol 4000 to 2 mL of final volume, and then centrifuged at $3000\times g$ for 12 hr at 4°C using Nalgene centrifuge tube. The solution obtained was used as the partially purified peach PPO for electrophoresis.

Polyphenol oxidase (PPO) assay

PPO activity of crude peach extract was determined according to the method described by Chang et al. (17). Crude enzyme solution (0.3 mL) was added to 0.03 M catechol (2.7 mL) as the substrate in 0.1 M sodium phosphate buffer (pH 6.2), and then determined absorbance at 420 nm. One unit of PPO activity was defined as an increase of 0.01 unit of absorbance per min at 420 nm.

Electrophoresis

Native polyacrylamide gel electrophoresis of partially purified PPO was performed using the Laemmli method without addition of SDS (21). The partially purified peach PPO (2 mL, 2 mg of protein/mL) was applied along the entire surface of a 4% stacking gel polymerized on top of a 7.5% separating gel. Upon completion of the electrophoresis, the gels were soaked in 0.1 M phosphate buffer (pH 6.8) for 10 min and cut into vertical strips. The strips were incubated with L-DOPA (final concentration 2 mM) for 30 min, and then visualized active band of PPO. In addition, the strips also were stained for protein with Coomassie brilliant blue R-250.

Statistical analysis

Values represent means of three replications, with a peach fruit serving as a replication. Statistical analysis was accomplished with the Statistical Analysis System (22) software package on replicated test data. Significant differences between means were determined by Duncan's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Physical characteristics of peach cultivars

Comparison of some physical characteristics of white- and yellow-fleshed peach cultivars is shown in Table 1. There is no significant difference in firmness and color values among white-fleshed peaches, except for "Yumyung", which had the strongest firmness with 58.2 N. Meanwhile, yellow-fleshed peaches had lower L^* and higher b^* (yellowness) value than that of white-fleshed peaches. In addition, it was found that "Changbang", "Keedo" and "Yumyung" were clingstone peaches, while "Daegubo" and "Hwangdo" were non-clingstone peaches. These findings indicate that a yellow-fleshed peach "Hwangdo" may be suitable as a raw material of processed peach products because it is a non-clingstone peach with a peculiar color.

Chemical characteristics of peach cultivars

Several chemical characteristics of white- and yellow-fleshed peach cultivars are compared in Table 2. Significant differences were found in chemical components of two peach cultivars. The soluble solids/titratable acidity ratios, L-ascorbic acid and total phenolic content were significantly higher for white-fleshed than those for yellow-fleshed cultivars. Meanwhile, there is no significant difference in the soluble solids,

Table 1. Physical properties of white- and yellow-fleshed peach cultivars harvested at optimum maturity

Type	Cultivar	Harvest date	Hunter value (L^* , a^* , b^*)	Firmness ¹⁾	Peach ²⁾
White-fleshed peach	Changbang	7/30	78.1 ^a , -2.0 ^a , 12.4 ^{ab3)}	45.8 ^a	Clingstone
	Daegubo	8/15	79.8 ^a , -2.1 ^a , 13.8 ^a	46.2 ^a	Non-clingstone
	Keedo	8/30	78.8 ^a , -2.3 ^a , 11.8 ^a	40.7 ^a	Clingstone
	Yumyung	9/05	79.2 ^a , -1.8 ^a , 11.5 ^a	58.2 ^b	Clingstone
Yellow-fleshed peach	Hwangdo	8/20	73.7 ^b , 0.7 ^b , 47.8 ^b	46.8 ^a	Non-clingstone

¹⁾Rheometer pressure values in Newtons (5-mm tip).

²⁾Clingstone and non-clingstone showed whether peach flesh and kernel are easily separated or not. Standard deviations have been omitted for simplicity.

³⁾Means in each column with same letter are not significantly different ($p<0.05$).

Table 2. Contents of chemical components of white- and yellow-fleshed peach cultivars harvested at optimum maturity

Type	Cultivar	Soluble solids ($^\circ\text{Brix}$)	Total acidity (%)	L-Ascorbic acid (mg/kg)	Total phenolics (mg/kg)
White-fleshed peach	Changbang	9.9 ^{a1)}	0.27 ^c	102 ^a	685 ^a
	Daegubo	9.6 ^a	0.28 ^c	120 ^a	702 ^a
	Keedo	10.9 ^a	0.24 ^c	119 ^a	747 ^a
	Yumyung	10.8 ^a	0.38 ^b	110 ^a	616 ^b
Yellow-fleshed peach	Hwangdo	7.5 ^b	0.48 ^a	79 ^b	328 ^c

¹⁾Means in each column with same letter are not significantly different ($p<0.05$). Standard deviations have been omitted for simplicity.

L-ascorbic acid and titratable acidity among white-fleshed peaches. One exception was "Yumyung", which showed the highest titratable acidity and the lowest phenolic content of all white-fleshed cultivars. These results support earlier reports that the white-fleshed peaches have greater levels of chemical components except total acidity than yellow-fleshed peaches (15,16), and their components in peaches varied with cultivar, maturity and storage (6,7).

Phenolic composition

An HPLC chromatogram of 80% methanolic extracts of white- and yellow-fleshed peaches is shown in Fig. 1. Flavan-3-ol, catechin, and hydroxycinnamates, neochlorogenic acid and chlorogenic acid were found as major phenolic components of two peaches. In the white-fleshed peaches, catechin was present in the highest levels, ranging from 52.58 to 101.65 mg/kg, followed by neochlorogenic acid, ranging from 32.19 to 81.53 mg/kg, and chlorogenic acid, ranging from 12.69 to 35.13 mg/kg, in descending order. Meanwhile, the yellowfleshed peach "Hwangdo" had higher neo-

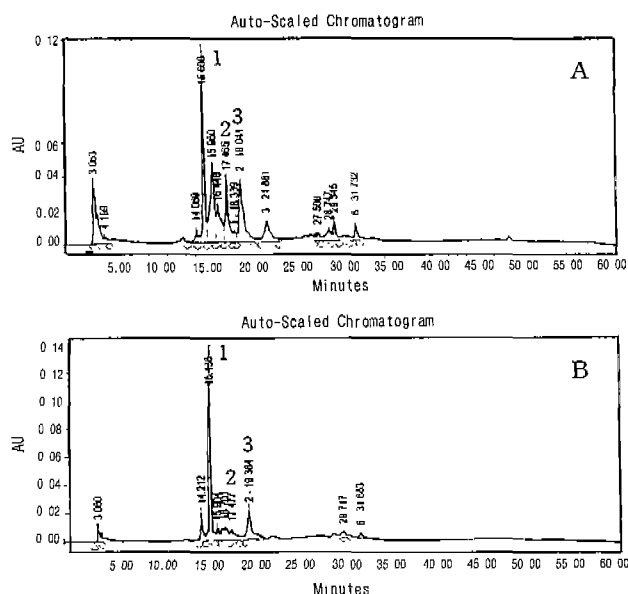


Fig. 1. HPLC chromatograms of phenolic compositions isolated from white-fleshed (A, Daegubo) and yellow-fleshed (B) peach cultivars. 1, Neochlorogenic acid; 2, catechin; 3, chlorogenic acid. HPLC conditions; column, μ Bondapak C18 (3.9×30 cm); gradient elution from solvent A (0.1% TFA in 5% MeOH) to solvent B (80% MeOH) for 50 min; flow rate, 1.0 mL/min; UV detection, $A_{280\text{nm}}$.

Table 3. Major phenolic compositions and polyphenol oxidase (PPO) of white- and yellow-fleshed peach cultivars harvested at optimum maturity (mg/kg, fresh weight)

Type	Cultivar	Neochlorogenic acid	Catechin	Chlorogenic acid	PPO ¹⁾ activity (units)
White-fleshed peach	Changbang	35.41 ^{b2)}	57.84 ^c	35.13 ^a	6.1 ^b
	Daegubo	81.53 ^a	101.65 ^a	12.69 ^b	8.9 ^a
	Keedo	42.20 ^b	74.25 ^b	31.86 ^a	6.8 ^b
	Yumyung	32.19 ^b	52.58 ^c	31.94 ^a	3.0 ^c
Yellow-fleshed peach	Hwangdo	46.67 ^b	22.90 ^d	16.07 ^b	3.3 ^c

¹⁾Polyphenol oxidase; one unit of PPO activity was defined as an increase of 0.01 unit of absorbance/min at 420 nm.

²⁾Means in each column with same letter are not significantly different ($p < 0.05$).

Standard deviations have been omitted for simplicity.

chlorogenic acid content (46.67 mg/kg) but less catechin (22.90 mg/kg) and chlorogenic acid (16.07 mg/kg) (Table 3). These findings were not in agreement with previous reports that chlorogenic acid was the predominant phenolic compound in clingstone peaches (15-17). Much higher levels of catechin and neochlorogenic acid were found in the "Daegubo" peach. "Changbang" contained the highest amount of chlorogenic acid of all peach extracts. Thus, these results support previous reports that the phenolic compositions and contents in peaches varied with varieties, maturity and ripening (15,16). Further characterization and quantification of other phenolic compounds in peaches are now being performed.

PPO activity and isozymes

PPO activity in crude extracts of both white-fleshed peaches and yellow-fleshed peach was given in Table 3. Significant differences in PPO activity were found among peach cultivars. Generally, the white-fleshed peaches had higher PPO activity than that of the yellow-fleshed peach, except for "Yumyung", which had lowest PPO activity of all peaches. Meanwhile, "Daegubo" had the highest PPO activity of all peach cultivars, whereas "Yumyung" and "Hwangdo" peaches had lower PPO activity than other peaches. These observations supported previous reports that the PPO activity of peaches varied with cultivars and maturity (14,16). Meanwhile, it is very interesting to note that "Yumyung" peach with the highest firmness (Table 1) had highest level of total acidity, and lowest total phenolics and PPO activity of all white-fleshed peaches. Thus, considering harvest date and quality characteristics of peaches, "Yumyung" peach cultivar can be used as potential source of dried peach fruit.

Meanwhile, the crude PPO extracts of white- (Daegubo) and yellow-fleshed (Hwangdo) peaches were partially purified by salting out with $(\text{NH}_4)_2\text{SO}_4$ and filtered with a membrane centrifuge tube, and then finally a partially purified PPO was performed by electrophoresis to detect PPO isozymes. As a result, three active bands of PPO including one major band with R_m 0.19 and two minor bands with R_m 0.38 and 0.50 were isolated from both the white- and yellow-fleshed peaches (Fig. 2), and were consistent with protein bands. There were no big differences in PPO isozyme patterns between two different peach cultivars. Further investigation on biochemical characteristics of three isozymes from peach cultivars is now in progress.

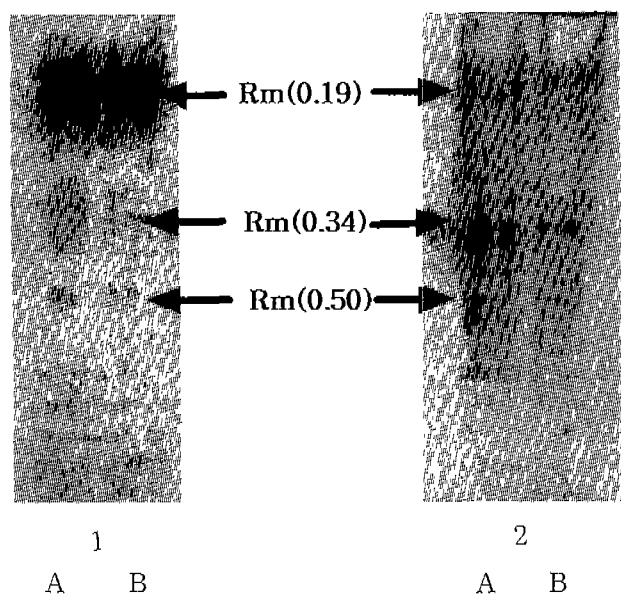


Fig. 2. Slab gel electrophoresis profile of partially purified PPO isozymes isolated from white-fleshed (A, Daegubo) and yellow-fleshed (B) peach cultivars. 1, Active bands; 2, protein bands. Electrophoresis was performed according to Materials and Methods.

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