Changes in Phenolic Acid Contents of *Diospyros lotus* L. during Fruit Development

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Changes in phenolic acid contents during fruit development of *Diospyros lotus* L. were studied. Salicylic, 4-hydroxybenzoic, vanillic, gentistic, 3,4-dihydroxybenzoic, syringic, *p*-coumaric, and gallic acids were identified and quantitated by gas chromatography. The phenolic acid contents, except gallic acid, increased in September, reached its highest value at the end of September, and then decreased rapidly. The fruits harvested in June, July, August, November, and October have phenolic acid contents significantly lower than fruits harvested in late September. Gallic, salicylic, and vanillic acids were predominant and present in all stages of development, except gallic acid, for the fruits harvested at the end of November.

Keywords: *Phenolic acids; Diospyros lotus; fruit development*

INTRODUCTION

Diospyros lotus L. (persimmon) is a deciduous tree, from the family Ebenaceae, which grows naturally in northeast and south Anatolia. It has been cultivated in northeast Anatolia for its edible fruits. The fruits are globes, 1.5−2 cm in diameter and bluish-black when mature (Davis, 1978). The fruits are normally not consumed directly after harvesting, because of their astringent taste, but are dried and sold in the markets. Preliminary reports are available in the literature on the sugar composition of the fruits (Ayaz et al., 1995), and it is reported that the fruits have a rich sugar content. In general, phenolic, nonvolatile, and organic acids have been widely studied in fruits (Ashoor and Knox, 1982; Bradoud and Pratz, 1986). Phenolic acids and their derivates are widely distributed in plants. Their levels varied dramatically, especially as influenced by factors such as germination, fresh, drying, and processing (Kakiuchi and Itoo, 1980). These are carbon-based compounds (Maga, 1978) which have been shown to have a role in tissue browning, flavor, and color characteristics of fruits and derived products (Appel, 1993) and implicated as possibly influencing the toxicological, nutritional, sensory, and antioxidant properties of foods (Ho et al., 1992; Hermann, 1990). Some species of *Diospyros* such as *D. kaki* and *D. virginiana* and its related cultivars have been studied for their soluble tannins, sugars, and nonvolatile acid compositions (Hiari and Yamazaki, 1983, 1984; Ishi and Yamanishi, 1982; Kakiuchi and Itoo, 1980; Senter et al., 1991). No studies on the changes of phenolic acids during fruit development of *D. lotus* have previously been reported. There is also no study on the phenolic content of persimmon grown in northeast Anatolia (Turkey). Research has been initiated to evaluate the chemical properties of persimmon grown in Turkey. We are reporting variations in phenolic acids in persimmon (*D. lotus*) during fruit development.

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Table 1. Collection Dates and Days of the Year of the Fruit Samples of *D. lotus* L.

harvest no.	harvest date	days of year	color of fruit
1	June 20, 1995	171	dark green
2	July 11, 1995	192	green
3	July 31, 1995	212	light green
4	August 21, 1995	233	yellow-green
5	September 10, 1995	253	yellow-green
6	September 30, 1995	273	yellow-green
7	October 20, 1995	293	yellow
8	November 10, 1995	314	yellow
9	November 30, 1995	334	yellow

MATERIALS AND METHODS

Sampling. Fruits from *Diospyros lotus* L. were harvested from various parts of the young trees during early morning at the Campus of Karadeniz Technical University. During each persimmon collection stage, 0.5 kg samples were collected randomly. Then the samples were dried under vacuo at 60 °C (Nüve EV 60). These samples were obtained on a continuing basis at 20 days intervals after flowering, from June to November 1995. Fruits were harvested in early morning and maintained below 12 °C until arrival at the laboratory.

Extraction of Phenolic Acids. Phenolic acids were extracted according to the method of Hanna et al. (1991). Five gram samples of powdered mesocarps of the dried samples were extracted exhaustively with petroleum ether (40−60 °C) to remove nonpolar compounds. The residue was then hydrolyzed with 50 mL of 2 N NaOH at room temperature under a nitrogen atmosphere for 4 h. The slurry was filtered and acidified with 10% HCl solution to pH 2.6 and extracted three times with 50 mL portions of ethyl acetate. Combined ethyl acetate extracts were dried over anhydrous magnesium sulfate and evaporated to dryness on a rotary evaporator under reduced pressure.

TMS Derivatization. The analytical procedures of Chapman and Horvat (1989) were used to prepare oxime TMS derivatives. The sample was filtered (0.45 μm filter), and an aliquot of 1 mL from each sample was dried under nitrogen and then silylated by adding pyridine and BSTFA (bis-(trimethylsilyl)trifluoroacetamide (1:1, v/v). The silylated extracts were analyzed by gas chromatography−mass spectrometry.

Gas Chromatography. TMS acids of the samples were analyzed in duplicate using a HP 5890-5970 GC−MS instru-

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Table 2. Variations in the Phenolic Acid Contents in *D. lotus* L. during Fruit Development[a]

compounds	days of year								
	171	192	212	233	253	273	293	314	334
salicylic acid	1.72^{e}	1.35^{c}	1.80^{f}	1.38^{d}	2.47^{g}	24.58^{i}	0.91^{b}	6.27^{h}	0.10^{a}
4-hydroxybenzoic acid	0.89^{g}	0.65^{e}	0.69^{f}	0.63^{d}	0.56^{c}	5.19^{i}	0.28^{b}	2.07^{h}	0.12^{a}
vanillic acid	2.31^{f}	1.61^{d}	1.81^{e}	1.55^{c}	11.4^{h}	16.20^{i}	0.89^{b}	2.99^{g}	0.22^{a}
gentistic acid	0.27^{d}	0.15^{b}	0.16^{bc}	0.18^{c}	0.39^{e}	8.96^{g}	0.16^{bc}	1.31^{f}	0.03^{a}
3,4-dihydroxybenzoic acid	9.78^{h}	0.86^{e}	0.86^{e}	0.42^{d}	0.35^{b}	3.24^{g}	0.18^{a}	1.92^{f}	0.37^{c}
syringic acid	0.56^{e}	0.28^{c}	0.40^{d}	0.38^{d}	0.40^{d}	6.31^{g}	0.26^{b}	2.00^{f}	0.07^{a}
p-coumaric acid	0.78^{e}	0.25^{b}	0.27^{c}	0.95^{f}	1.46^{g}	15.49^{i}	0.64^{d}	7.95^{h}	0.13^{a}
gallic acid	10.91^{c}	54.11^{i}	40.68^{g}	33.46^{f}	14.43^{e}	11.46^{d}	6.90^{b}	48.06^{h}	0.68^{a}

[a] Means of three replications. Values with the same letter are not significantly different at $p = 0.05$. The means were compared within each row of the data, not columns.

ment. The column was a HP-1 capillary column (25 m × 0.32 mm i.d., 0.17 μm film thickness) with a flow rate of 3.0 mL/min, and the initial oven temperature was 60 °C. After 2 min, the oven temperature was increased at a rate of 8 °C/min for 12 min and held at 250 °C for 3 min. Helium was used as the carrier gas at a flow rate of 50 cm/s. The quantitative calculations were based on the peak area from GC-FID. Peak areas were measured with a Merck-Hitachi D-2000 integrator. Authenticity of identification by mass spectral analyses was reported by Chapman and Horvat (1989). Mass spectrometer conditions were selected are as follows: ion source temperature, 150 °C; scan rate, 200 amu/s; ionization energy, 70 eV. The interface temperature between the GC and mass spectrometer was 200 °C. The quantity of phenolic acids was in relation to an internal standard after computation of appropriate response factors and was reported in units of milligrams per gram of dry matter. Individual phenolic acids were identified by comparison of retention times and mass spectra with those of TMS derivates of their authentic compounds prepared in the same manner.

Statistical Analysis. The extraction and determination of phenolic acids were replicated three times. Analyses of variance of the data were evaluated by the Statistical Analysis System. Duncan's Multiple Range Test was employed to determine the statistical significance of differences among the means.

RESULTS AND DISCUSSION

Collection dates and days of the year of the fruit samples of *D. lotus* are shown in Table 1.

To prevent possible variations due to the different water contents (87.4−95.3%) of the samples, all phenolic acid contents were reported as dry matter units. The values obtained showed significant differences among the mean concentrations of each harvesting stage (Table 2). The phenolic acids idendified and quantitated in developing fruits of *D. lotus* were salicylic, 4-hydroxybenzoic, vanillic, gentistic, 3,4-dihydroxybenzoic, syringic, *p*-coumaric, and gallic acids. Gallic acid is the most abundant, and salicylic, vanillic and *p*-coumaric acids are the second most abundant phenolic acids during the fruit development (Table 2).

Since differences in phenolic acid contents were observed in some cases among samples harvested during the same month, it was possible to see a change in the phenolic acid contents from the beginning to the end of the season.

The phenolic acid contents of harvested *D. lotus* fruits quantitated lower in June, July, and August until the beginning of September. *D. lotus* fruits harvested at the end of September have phenolic acid contents higher than the ones of June, July, and August, except gallic acid. Gallic acid has the richest content at the beginning of July (in day 192) and at the beginning of November (in day 314). Statistical analysis in Table 2 indicates significant variation ($p < 0.05$) in quantities of all phenolic acids during the development stage of the fruits.

The data obtained show that the phenolic acid contents of *D. lotus* are lower from the beginning of the development until day 253. The content of the gallic acid increased rapidly after day 171 (54.11 mg/g of dry matter in day 192) and then decreased gradually until day 293. A very sharp rise was followed in day 314 and then remained very low after day 334 and then not determined after day 334. Rapid increases of phenolic acids were obtained after day 233, but the contents are much lower than those at day 273, except gallic acid. The acid, in contrast to the other acids, increased rapidly after day 293 (48.06 mg/g of dry matter in day 314). Progressive increases and decreases in the contents of phenolic acids occurred with development of fruit in all stages in which they remained much lower during ripening (post ripening) after days 314 and 334. Fruit color might simply be an indicator of its developmental process. According to the data in Table 2, it can be said that the growing stage continues up to day 273, then the maturation stage starts. Thus, a gradual increase of phenolic acids until day 273 and then a decrease can be explained by growth, maturation, and postmaturation (senescence) processes of the fruit. Similar results have been given in the literature. For example, it has been reported that the level of total phenols, tannins, and condense tannins were substantially lower in the young plant, suggesting that these constituents increase with maturity of the plant. But the senescence process decreases the level of tannins, as is evident from low values for the dry, dead fallen stem (Makkar et al., 1993). Similar results were also obtained for fallen oak leaves (Makkar and Singh, 1991; Makkar et al., 1991)). However, to explain the changes of gallic acid in the fruit during the stages of development is very difficult.

Not all of the samples show a regular behavior that makes it possible to easily identify it with the data given in Table 2. This is due to the fact that the fruits are living materials and therefore subject to physiological changes that are a function of several factors (origin, variety, development stage, temperature, etc.).

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