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Research Article

Nonvolatile Acid Composition During Fruit Development of *Diospyros lotus* L.

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Abstract: Changes in nonvolatile acid contents during fruit development of *Diospyros lotus* L (*Ebenaceae*) were studied. Succinic, furmaric, malic, citric and azelaic acids were identified and quantified by gas chromatography. The quantities of all acids varied significantly during the fruit development. Malic and citric acids were generally predominant in all development stages. During havesting time (on day 331), fumaric acid was the most abundant acid in the fruits. Quantities of malic, succinic, fumaric and citric acids (except azelaic acid) were found to be the highest in September (on day 271).

Key Words: Nonvolatile acids, *Diospyros lotus*, fruit development.

Diospyros lotus L.'dan Meyva Gelişimi Boyunca Uçucu Olmayan Asit Kompozisyonu

Özet: *Diospyros lotus* L.'un (*Ebenaceae*) uçucu olmayan asitlerinin içeriğindeki değişimler çalışıldı. Gaz kromatografisi ile süksinik, fumarik, malik, sitrik ve azelaik asitler belirlendi ve miktarları tayin edildi. Bütün asitlere ait miktarların meyva gelişimi sırasında önemli derecede değişiklik gösterdiği bulundu. Malik ve sitrik asit bütün gelişme safhalarında en fazla bulunan asitler idi. Fumarik asitin diğer asitlere oranla meyvaların normal hasat zamanında (331. gün) en bol bulunan asit olduğu tespit edildi. Malik, süksinik, fumarik ve sitrik asit (azelaik asit hariç) en yüksek miktarlarda Eylül ayında (271. günde) bulundu.

Anahtar Sözcükler: Uçucu olmayan asitler, *Diospyros lotus*, meyva gelişmesi

Introduction

Diospyros lotus L. is a deciduous tree up to 15 m in height, from the family of *Ebenaceae*, which grows naturally in Northeast and South Anatolia. It was cultivated in Northeast Anatolia for its edible fruits. The fruits are globose, 1.5-2.0 cm in diameter and yellow or bluish-black in colour in maturity (1). The fruits are preferably not eaten in immature form because of their astringent taste. Fresh and mature fruits or dried ones are eaten and sold in the markets. The fruits of this species are also used in folk medicine in Turkey as a treatment for constipation (2).

Nonvolatile acids are natural components of many fruits and vegetables (3). One class of fruit components, organic acids, plays an important function in maintaining fruit quality and nutritive value, for example in apples (4). Malic acid accounts for about 90% of the acid content of apples while citric, succinic, and traces of several other acids make up the rest (5).

Most information on the fruits of *D. lotus* is related to astringency removal, (6), sugar composition (7), phenolic acid changes during fruit development (8), and physical and biochemical properties (9). However, no studies have previously been done on the changes of individual nonvolatile acids during fruit development and maturation of *D. lotus*. The importance of these compounds is especially pertinent in assessing factors contributing to the flavor characteristics of persimmons (*Diospyros* L.). The purpose of this study was to monitor the changes of nonvolatile acids during nine stages of fruit development in *D. lotus*.

Materials and Methods

Fruit sample: Fruits from *D. lotus* were harvested from young trees on the Campus of Karadeniz Technical University. During each stage, 0.5 kg persimmon samples were collected randomly. These samples were obtained on a continual basis at 20-day intervals fol-

Harvest no.	Harvest date	Days of year	Colour of fruit
1	June 20, 1995	171	Dark green
2	July 10, 1995	191	Green
3	July 30, 1995	211	Light green
4	August 19, 1995	231	Yellow-green
5	September 8, 1995	251	Yellow-green
6	September 28, 1995	271	Yellow-green
7	October 18, 1995	291	Yellow
8	November 7, 1995	311	Yellow
9	November 27, 1995	334	Yellow

Table 1. Collection dates and numbered days of year of the fruit samples of *D.lotus* L.

lowing flowering, prior to defoliation, from June until November, 1995. Fruits were harvested in the early morning and maintained below 12°C until arrival at the laboratory. The mesocarps of the fruits were removed from the seeds and dried in vacuo overnight. The dried material was ground with a Waring Blender and stored below 0°C in a refrigerator until use.

Extraction of Nonvolatile Acids: Nonvolatile acid extraction was done according to Senter et al. (10). According to the procedure, 20 g of prepared mesocarps were extracted with 50 ml 70% ethanol in a screw-capped container by shaking 10 min with a wrist-action shaker. The extract was filtered through a Whatman no. 4 filter paper and again 50ml extracting solvent was used. Aliquots of 0.5 ml of these extracts were used for the acid analysis.

Trimethysilyl (TMS) Derivatization: The analytical procedures of Chapman and Horvat (11) were used to prepare oxime-TMS nonvolatile acids. A test tube containing 0.5 ml of extract was placed on a heating block at 75°C and the solvent was evaporated with a stream of dry nitrogen. Then the acids were converted to TMS derivatives by adding 0.5 ml of BSTFA [bis (trimethylsily)trifluoroacetamide] + 1 % TMCS (trimethylchlorosilane) (Fluka Chemical Co.) and heating additional 20 min at 75°C. Following the reaction, a small amount of anhydrous sodium sulphate was added to ensure dryness.

Gas Chromatography: TMS acids of the prepared samples were analyzed in duplicate using an HP 5890-5970 GC-MS instrument. The column was a HP-1 capillary column (25 m x 0.32 mm i.d., 0.17 µm film thickness). Injector and detector temperatures were 225 and 280°C respectively. Oven temperature was held at 150°C for 4 min, then programmed to 192°C at 4°C/min and maintained for 0.5 min, and then programmed to 240°C at 10°C/min and maintained for 7 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. A similar column, with helium as carrier gas, was used under the same conditions in gas chromatography-mass spectrometry analysis (HP 5890-5970 GC-MS): ion source temperature, 150°C; scan rate, 200 amu/s; ionization energy, 70 eV. The interface temperature between the GC and the mass spectrometer was 200°C. Individual acids were identified by comparison with their 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 nonvolatile acids were conducted three times. Analysis of variance of the data was evaluated by the Statistical Analysis System. Duncan's Multiple Range Test was employed to determine the statistical significance of differences in the means.

Results and Discussion

Harvesting dates and numbered days of year of the fruit samples in *D. lotus* fruit are show in Table 1. The values obtained showed significant differences in the mean concentrations of the harvesting stage (Table 2). All nonvolatile acid contents are reported as dry matter units.

The nonvolatile acids identified and quantified in developing fruits of *D. lotus* were succinic, malic, fumaric, azelaic and citric acids (Table 2). Malic acid was the most abundant, and fumaric and citric acids were

Table 2. Variations in the nonvolatile acid compositions in *D.lotus* L. during fruit development (mg/g dry weight).

	Days of year								
Compounds	171	191	211	231	251	271	291	311	331
Succinic caid	0.20b*	0.30d	0.27c	0.22c	0.2b	3.43g	0.93f	0.78e	0.10a
Fumaric acid	4.50g	3.40e	4.20f	0.51a	1.34c	14.70i	10.43h	3.10d	0.70b
Malic acid	4.90d	6.43e	9.71f	2.61c	1.90b	30.03i	22.10h	10.60g	0.16a
Azelaic acid	0.13c	0.07a	0.08ab	--**	0.09b	--	0.07a	3.00c	0.20d
Citric acid	3.00e	0.80b	1.73c	2.30d	2.43d	38.12h	23.00g	17.00f	0.50a

* Values, means of three independent determinations. Values with the same letter are not significantly different at p=0.05 (The means were compared by row of the data, not column)

** not detected

next most abundant nonvolatile acids during fruit development (Table 2). Traces of azelaic acid were observed on days 231 and 271. The succinic acid level remained approximately constant until day 251, increased rapidly on day 271 and then declined gradually until day 331. Decreases in fumaric, azelaic and citric acids were also observed between days 171 and 251 in comparison with their initial levels. The malic acid level showed an unstable change until day 251. The highest level of this acid was found on day 271, and then the level decreased gradually until day 331. The maximum levels of succinic, fumaric, citric and malic acids were observed on day 271, but ozealic acid was not detected at this stage. After day 271, the levels of these acids declined steadily, reaching minimum levels. An increase was observed in azelaic acid level on day 311, followed by a sharp decrease on day 331.

Fruit colour might simply be an indicator of its developmental process. Thus, according to the data in Table 2, it can be said that growing stage continues up to day 271, then the maturation stage starts. Thus, there was a gradual increase in nonvolatile acids until day 271, followed by a decrease, which can be explained by growth, maturation and post-maturation (senescence). Similar results were also obtained from preliminary studies. In a peach cultivar (cv.Monoroe) during maturation between days 81 and 143 after flowering, the main nonvolatile acids were found to be malic, citric and quinic acids. The highest levels of citric and malic acids were observed as 0.7% fresh weight on day 110, and 0.6% fresh weight on day 130, respectively. In midripe and ripe stages between days 130 and 140 the levels of these acids decreased to their minimums, comprising 0.10% of fresh weight for citric and 0.30% of fresh weight for malic acid (12). These researchers have done similar studies on mayhaw fruits (*Crataegus* L.). The levels of nonvolatile acids decreased in significant amounts from midripe to ripe stages (13).

Ackerman et al. (4) have observed the changes in the content of malic and citric acids in an apple cultivar (cv.Glockenapfel) during ripening from the first to the 25th week on a continuing basis at 3-week intervals. In the fruits, the highest levels of malic acid per fresh weight were observed in the 5th week (1.2%) and in the period between the 15th and 18th weeks (0.8%). Citric acid showed its highest levels in the 5th week (8.0%) and in the 15th week (6.0%) and then decreased to 4.0% after 15th week.

Succinic, malic, citric and quinic acids were identified as the major nonvolatile acids during five stages of maturation in the fruits of five cultivars of *Diospyros kaki* L. In the Fuyu cultivar, the highest levels of succinic and malic acids were found at the third stage of maturation, 0.45 and 0.83 g/100 g dry matter, respectively, while citric acid was found in the first stage of maturation. In contrast to the Fuyu cultivar, the highest levels of succinic and malic acids were found at the 5th stage of maturation in the Giambo cultivar. The cultivars Ichi Kijiro and Aizumi Shiraza showed high levels of succinic, malic and citric acids between the first and second stages of maturation (10).

The results showed that the highest content of succinic, fumaric, citric and malic acids were found on day 271, principally. As indicated above, these increases might be explained by the start of the maturation process. In a more recent study of the fruit of the same plant, similar results have been reported (8). For example, as in the case of our study, the highest and the lowest levels of phenolic acids during fruit development of *D. lotus* were observed on days 271 and 331, respectively. In addition, the highest levels of fructose and glucose were obtained at days 271 and 291, respectively (unpublished research).

On the basis of this study, the main nonvolatile acids (malic, citric and fumaric acids) were found to reach their high levels the between the end of September and the beginning of November. Time could be used as a reliable index for physiological maturity in *D. lotus* fruits. A more detailed knowledge of the chemical compositional changes in the fruits could be a desirable feature for selecting *D. lotus* with improved nutritional quality.

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