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CHANGES OF MAJOR PHENOLIC COMPOUNDS, MAJOR CAROTENOIDS AND L-ASCORBIC ACID COMPOSITION DETERMINED BY HPLC IN PERSIMMON *(Diospyros kaki L.)* DURING RIPENING

TRABZONHURMASININ *(Diospyros kaki L.)* OLGUNLAŞMA SÜRECİNDE HPLC İLE BELİRLENEN MAJOR FENOLİK BİLEŞİK, MAJOR KAROTENOİD BİLEŞİK VE L-ASKORBİK ASİT KOMPOZİSYONUNUN DEĞİŞİMİ

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ABSTRACT: In this study, major phenolic compounds, major carotenoids and L-ascorbic acid change of fuyu (non-astringent) and *hachiya* (astringent) cultivars of persimmon were studied by HPLC during ripening period. Especially in *hachiya* cv, known to be astringent, phenolic compounds decreased from 3747.0 mg/kg to 132.05 mg/kg at the end of ripening. The highest value of phenolic compounds jwas 84.7 mg/kg during ripening, decreasing to 39.6 mg/kg for *fuyu* cv. Carotenoids steadily increased to 36.5 mg/kg and 73.5 mg/kg at the end of ripening in *fuyu* and *hachiya* cvs, respectively. L-ascorbic acid amount decreased to 15.4 mg/kg in *fuyu* cv but increased to 15.9 mg/kg in *hachiya* cv at the end of ripening.

ÖZET: Bu araştırmada trabzonhurmasının *fuyu* (buruk olmayan) ve *hachiya* (buruk) çeşitlerinin olgunlaşma periyodunda ana fenolik bileşik, ana karotenoid bileşik ve L-askorbik asit değişimi HPLC ile belirlenmiştir. Özellikle buruk olan *hachiya* çeşidinde olgunlaşma sırasında fenolik bileşikler önemli miktarda azalma göstermiştir; en yüksek 3747.0 mg/kg değerinden 132.05 mg/kg değerine düşmüştür. Bu değerler *fuyu* çeşidinde ise en yüksek 84.7 mg/kg bulunurken olgunlaşma sonunda 39.6 mg/kg düzeyine kadar düşmüştür. Toplam karotenoid bileşik miktarı ise artış göstermiş ve *fuyu* ve *hachiya* çeşitlerinde sırasıyla 36.5 mg/kg ve 73.5 mg/kg değerlerine ulaşmıştır. L-askorbik asit miktarı olgunlaşma sonunda *fuyu* çeşidinde azalmış (15.4 mg/kg) *hachiya* çeşidinde ise artmıştır (15.9 mg/kg).

INTRODUCTION

Persimmon (*Diospyros kaki* L.) is widely grown in many regions of the world (ITO, 1971). China and Japan are known to be the land of origin and big producers and consumers of persimmon. Fuyu and hachiya cvs are very popular in Turkey as in the world. Althought, it is not known when persimmon have cultured first in Turkey, it has a production total of 10.000 tons per year in Mediterranean and Black Sea regions (ONUR, 1990; ANONYMOUS, 1997).

In some investigations, conducted on deastringency of astringent cvs, unripe fruits were harvested and role of CO_2, ethylene and acetylene in the removal of astringency were researched under controlled atmosphre during maturation. When these component's ratio and storage temperature increased, fruits got ripen in a shorter time (PESIS et al., 1986). Despite the more intensive colour and shorter ripening time (KATO, 1990), fruit flesh changed to brown because of high acetaldehyde accumulation (PESIS et al., 1988). Also, carotenoids, reducing sugar and soluble solid contents increased in CO_2 applied samples during storage (TÜRK, 1993).

Table 1. Physical and Chemical Differences Between Astringent and Nonastringent CVS of Persimmon (FORBUS et al., 1991)

Property	Astringent cv	Non-astringent cv
Firmness (Newton)	57.6	74.8
Colour (Hunter a)*	12.6	12.9
Soluble solid (%)	18.9	15.1
β-carotene (µg/g)	212.0	265.6
Chlorophyll (µg/g)	99.6	118.5

FORBUS, et al. (1991) conducted some studies to find out relations between fruit firmness and ripening, physical and chemical differences between astringent and nonastringent cvs. (Table 1) and they reported that colour and firmness values were able to determine the ripening time.

Persimmon is known as a good source of L-ascorbic acid. L-ascorbic acid amount is higher in unripe fruits than ripen ones and it becomes higher from centre to peel of the fruits (ITO, 1971).

It is not a widespread application to ripe persimmon on trees before harvesting, because of fruit's ripening is not homogeneous and ripen fruits lose their firmness. But, we thought that it could be possible to ripe the persimmons on trees if changes of ripening criteria were controlled.

MATERIAL AND METHODS

Material

Samples were obtained from "Antalya Citrus and Greenhouse Research Institute" farm field for five weeks on a continuing basis at one week intervals from the second week of September, 1997. All samples were processed into pulp and homogenised then stored at-18°C in a deep-freeze.

Methods

Chemical Analyses

Total dry matter and total acidity (as citric acid) were defined according to CEMEROĞLU (1992); soluble solid was determined by Abbe Refractometer (ANONYMOUS, 1968a); pH value was determined as potentiometric method (ANONYMOUS, 1968b).

HPLC Determination of Phenolic Compounds

Ten (10) g of pulp was put into a 50 ml flask and 40 ml of water was added. After homogenised and centrifuged at 3.000 rpm, supernatant was filtered through a 0.45 µm membrane. 20 µl of filtrate was injected to HPLC system (SPANOS and WROLSTAD, 1990; ARTIK and MURAKAMI, 1997). HPLC system was included VARIAN LC Star Solvent Delivery System, UV-VIS detector (280 nm), Nucleosil 5 C18 column (250x4.6 mm, ID). Mobile phase was contained 5% formic acid and methanol (gradient). Flow rate was 1 ml/min (MAZZA and VELİOĞLU, 1992).

HPLC determination of L-ascorbic acid

60 g of pulp was taken and fixed to 250 ml with 6% HPO_3 containing 10^{-6} M EDTA and 10^{-7} M diethyl dithiocarbarbamic acid. The mixture was homogenised and centrifuged at 3.000 rpm for 30 min. The supernatant was filtered through the 0.45 µm membrane filter before 20µl injection (WATADA, 1982). A VARIAN LC Star System including UV-VIS detector (264 nm), Nucleosil 5 C18 column (250x4.6 mm, ID) was used. Mobile phase was 1.5% $NH_4H_2PO_4$ and flow rate was 0.9 mL/min.

HPLC determination of carotenoids

5g of pulp was transferred to 50 ml flask with 20 ml mixture of hexane: ethanol (1:1) and then homogenised. Then, mixture was transferred to a seperatory funnel and extracted with 20 ml hexane for 3-4 times until the residue turned colourless. The combined hexane extract was washed three times with water, collected, dried over Na_2SO_4 and concentrated in a rotary evaporator (40°C), the volume being adjusted to 25 ml with acetone. Acetone extract obtained was filtered through a 0.45 µm mmbrane and injected volume was 20 µl (WILBERG and DELIA, 1995) HPLC system included VARIAN LC Star Solvent Delivery System, UV-VIS Detector, Nucleosil 5 C18 column. Flow rate was 1 ml/min. The mobile phase was a mixture of acetonitrile and chloroform (92:8).

RESULTS AND DISCUSSION

Total dry matter, soluble solids, total acidity and pH value were significantly different ($p<0.01$) between both cvs according to chemical composition of fuyu and hachiya cvs shown in Table 2.

Table 2. Some Chemical Compounds of Persimmon

Component	Cultivar	
	Fuyu	Hachiya
Total dry matter (%)	19.3 B	22.4 A
Soluble solid (%)	16.5 B	19.0 A
Total acidity (g/kg)	0.9 B	2.3 A
pH value	5.3 B	5.6 A

The values have different letters in the rows were different ($p<0.01$).

Changes of L-ascorbic acid major carotenoids and major phenolic of both cvs are shown in Table 3 and asample from each of their chromatograms is presented in Figure 1, Figure 2 and Figure 3.

L-ascorbic acid level decreased during ripening though there was not a statistically significant change ($p<0.05$). But it increased significantly and regularly in hachiya cv. However, L-ascorbic acid amount of fruits generally shows a decreasing trend during ripening. In addition, a significant difference was found between L-ascorbic acid contents of both vcs at the beginning of ripening.

Table 3. Changes of L-Ascorbic Acid Major Carotenoids and Major Phenolic Compounds of Persimmon During the Five Stages of Ripening Period

Stages	L-Ascorbic acid (mg/kg)		Carotenoids (mg/kg)						Phenolic compounds (mg/kg)					
			Xantophyl		β-Carotene		Total carotenoids		Catechin		Quercetin		Total phenolic compounds	
	Fuyu	Hachiya	Fuyu	Hachiya	Fuyu	Hachiya	Fuyu	Hachiya	Fuyu	Hachiya	Fuyu	Hachiya	Fuyu	Hachiya
	AB	B		B		B	B	B		B		A		B
1	16.2	14.9	12.5	15.5	15.0	26.5.5	27.5	42.0	83.6	2135.5	0.0	361.5	83.6	2496.0
	a	b			b	a	b	a	b	a	b	a	b	a
	A	AB		B		C	AB	C		A		A		A
2	16.3	15.1	15.0	13.5	16.5	21.5	31.5	35.0	84.7	3398.1	0.0	348.9	84.7	3747.0
	a	b			b	a			b	a	b	a	b	a
	AB	AB		B		B	AB	BC		A		B		A
3	15.7	15.2	11.0	14.0	21.5	25.0	32.5	39.0	71.1	3526.0	0.0	219.4	71.1	3745.4
					b	a	b	a	b	a	b	a	b	a
	AB	AB		B		B	A	B		B		A		B
4	15.8	15.5	12.0	15.5	24.0	28.0	36.0	43.5	47.4	2169.6	0.0	326.9	47.4	2496.5
					b	a	b	a	b	a	b	a	b	a
	B	A		A		A	A	A		C				C
5	15.4	15.9	11.5	28.0	25.0	45.5	36.5	73.5	39.6	132.0	0.0	0.0	39.6	132.05
			b	a	b	a	b	a						

The values have different letters (caps) in the same column were different
The values of the same property have different letters (small) in the rows were different.

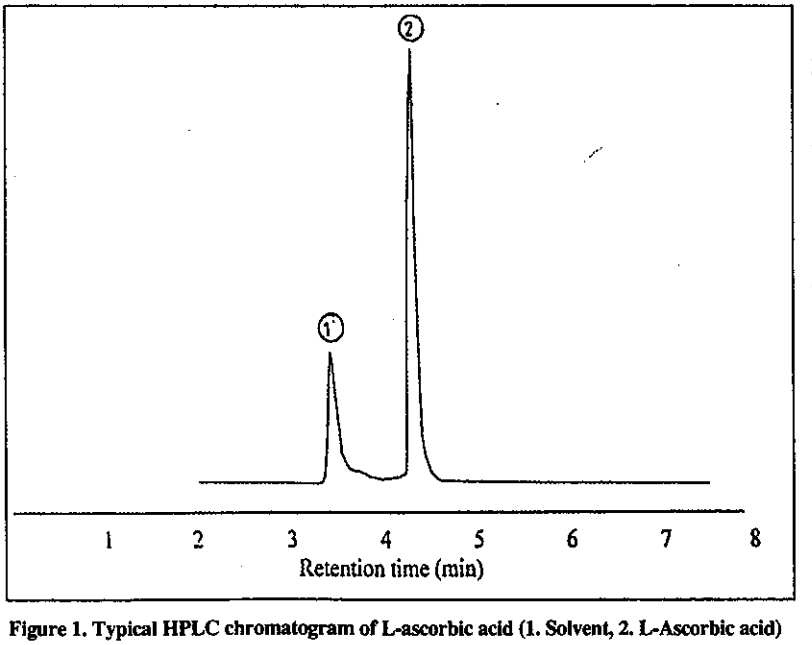

Figure 1. Typical HPLC chromatogram of L-ascorbic acid (1. Solvent, 2. L-Ascorbic acid)

ITO (1971) reported that persimmon had contained lycopene, β-carotene, zeaxantin, antheraxatin and neoantin to be dominant, also other carotenoids had been present in smaller amount. We found xantophyl and β-carotene as the major, and other caroteoids as small as not to describe. Xantophyl amount of fuyu cv did not change significantly ($p<0.05$), but hachiya cv, except for th last week of ripening period. β-carotene content was significantly different in both cvs during ripening period ($p<0.01$). Fuyu and hachiya cvs contained 36.5 mg/kg and 73.5

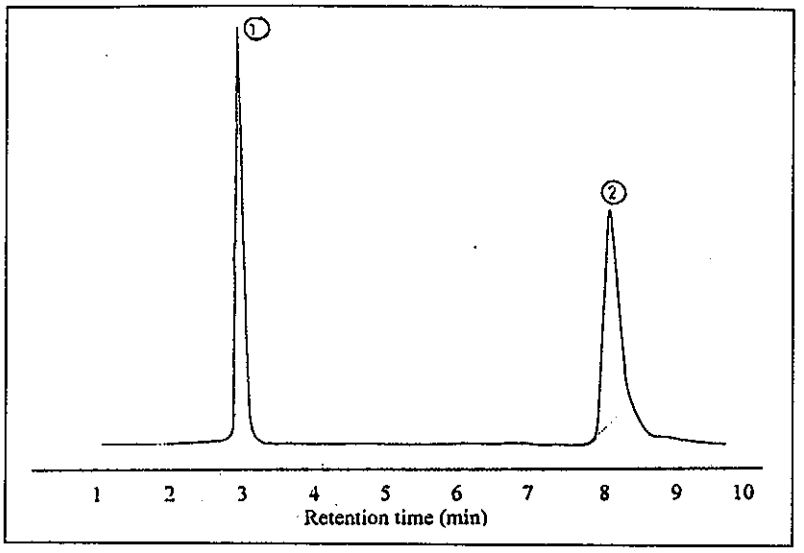

Figure 2. Typical HPLC chromatogram of major carotenoids (1. Xantophyl, 2. β-Carotene)

mg/kg total carotenoids, respectively in ripen fruits.

Catechin, from the flavonoid group of phenolic compounds, was found in both cvs but quercetin was only present in hachiya cv during four stages of ripening period. We thought that these components were the cause of significant astringency. Catechin content changed in hachiya cv but in fuyu cv ($p<0.01$). Quercetin which showed a significant decrease in hachiya cv was absent at the end of ripening period. Also, total phenolic compounds was not significantly different in booth cvs at the end of ripening ($p<0.01$). These results explained how much phenolic compounds effect the astringency. Thus, fuyu fruits can be eaten unripe, however catechin as present in this cv in all stages and it decreased by the time. So, it could be thought that catechin concentration as in fuyu cv is not cause of astringency, but fuyu cv did not contain quercetin over stages of ripening. In spite of hachiya containing quercetin during four stages of ripening, it was not certain which phenolic was the main source of astringency since hachiya did not contain ever a little amount of quercetin during the last stage.

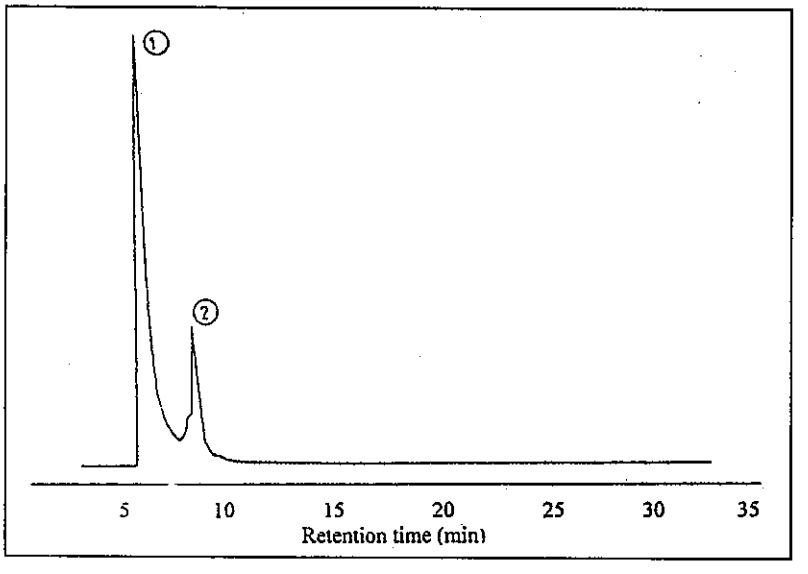

Figure 3. Typical HPLC chromatogram of major phenolic compounds (1. Catechin, 2. Quercetin)

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