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CAROTENOID CHANGES IN THE PEEL OF RIPENING PERSIMMON (*DIOSPYROS KAKI*) CV TRIUMPH

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Key Word Index—*Diospyros kaki*; Ebenaceae; persimmon; carotenoids; fruit ripening.

Abstract—The changes of the carotenoid pigments in the peel of ripening persimmon (*Diospyros kaki*) cv Triumph were followed for an entire season. During ripening, the total carotenoid decreased until colour break, then increased gradually and drastically at the last ripening stages. The chloroplast carotenoid pattern of the unripe fruit changed into a chromoplast pattern in which cryptoxanthin was the predominant pigment, reaching a level between 40 and 50 % of the total carotenoids. It accumulated continuously at a rate of approximately 10 % at each 2 week interval, its percentage being characteristic for each ripening stage. Other major pigments at levels of approximately 10 % of the total carotenoids were zeaxanthin, antheraxanthin and violaxanthin. In the fully ripe fruit, ripened both on and off the tree, lycopene which was not present before was found as the second major pigment. This unusual pattern change is discussed.

INTRODUCTION

The Japanese persimmon (*Diospyros kaki*), considered native to Japan, is the most important of the known persimmon species. It has at least one thousand varieties which are divided into groups according to colour and astringency [1].

The colour of the ripe fruit, from yellow-orange to red, is due to the carotenoid pigments of which the fruit is a rich source. They were first analysed in 1932 by Karrer *et al.*, who identified lycopene, zeaxanthin and 'various pigments' [2]. It was also reported that zeaxanthin is the first major pigment and cryptoxanthin the next [3]. A Japanese investigation of two persimmon varieties attributed the marked colour intensification, as ripening is completed, to a large increase in lycopene, up to 30–40 % of the total carotenoids [4].

In a detailed investigation of the Hachiya variety using the countercurrent distribution method of Curl [5], 23 carotenoids were isolated, excluding *cis*-isomers. The major pigments were found to be cryptoxanthin (38 %), zeaxanthin (18 %) and antheraxanthin (10 %). Lycopene amounted to only 8 %.

A comprehensive survey of carotenoid distribution in 40 varieties of Japanese persimmons revealed that cryptoxanthin was the major constituent, amounting to 30–35 % of the total carotenoids. The hydrocarbon fraction was found to be highly variable; in particular, lycopene content varied from 0 to 30 %. The levels of lycopene were not related to total carotenoid content. With respect to their lycopene content, the investigated varieties were classified into three categories: high lycopene (20–40 %), intermediate and low lycopene groups [6].

In the present study the carotenoids of cv Triumph grown in Israel were investigated for the first time. The changes in the carotenoid pattern were followed during the entire season in the peel, which is the region of highest carotenoid concentration in fruits.

The present report is one of a series of studies of pigment changes during ripening designed to provide a better understanding of carotenogenesis [7–11].

A problem of particular interest is the high variation of lycopene levels in the various persimmon cultivars [6].

RESULTS

Table 1 gives the quantitative changes of chlorophyll and total carotenoids and the changes of the carotenoid pattern of the peel of ripening persimmon.

Chlorophyll content decreased steadily during ripening and disappeared totally in the harvest-ripe fruit. The total carotenoid content decreased from 96.4 to 45.6 μg/g fr. wt, increased to 92 μg/g in the harvest ripe fruit, and reached the very high level of 310 μg/g fr. wt in the fully ripe fruit.

In the unripe fruit the chloroplast–carotenoid pigments were predominant; namely β-carotene, lutein, violaxanthin and neoxanthin. Additional minor pigments which may be found in chloroplasts were also present: α-carotene, α- and β-cryptoxanthin, isolutein, zeaxanthin and antheraxanthin.

As the fruit ripened the chloroplast carotenoids gradually decreased and typical chromoplast carotenoids such as cryptoxanthin, antheraxanthin and zeaxanthin were continuously synthesized, becoming major pigments.

A noteworthy feature was the constant increase of the cryptoxanthin level, which rose from an initial value of 1.7 to 42 % at harvest (stage VI) at a uniform rate increase of 8–10 % at each 2 week interval.

The fully ripe fruit (stage VII) underwent pronounced changes. A massive carotenogenesis produced a three-fold increase of the total carotenoid content and simultaneously a drastic change of the carotenoid pattern, lycopene becoming a major pigment. This completely changed the relative proportions of the individual carotenoids, lycopene representing 20 % and cryptoxanthin decreasing from 41 to 22 %.

Additional small amounts of precursors of the early

Table 1. Pigment changes in the peel of persimmon (*Diospyros kaki*) cv Triumph during ripening

		Ripening stages						
		I	II	III	IV	V	VI	VII
Chlorophyll a	μg/g fr. wt	127.7	82.5	50.2	43.8	17.2	—	—
Chlorophyll b		31.6	21.5	13.2	12.1	5.0	—	—
Total carotenoids		96.4	70.4	45.6	58.3	66.2	92.0	310.0
Carotenoid pattern (% of total carotenoids)								
Phytofluene		—	—	—	—	—	—	1.2
α-Carotene		2.6	3.2	2.9	2.6	2.0	1.8	1.0
β-Carotene		17.9	16.7	13.8	11.3	9.3	9.7	3.4
ζ-Carotene		—	—	—	—	—	—	0.2
Mutatochrome		—	—	0.7	—	—	0.8	—
γ-Carotone		—	—	—	—	0.5	0.5	3.2
Lycopene		—	—	—	—	—	—	19.8
α-Cryptoxanthin		1.1	—	—	—	—	—	—
β-Cryptoxanthin		1.7	6.8	12.7	24.6	32.8	41.7	21.8
Cryptoxanthin 5,6-epoxide		—	—	—	1.1	1.4	2.3	2.2
Cryptoflavin		—	—	—	—	—	—	1.0
Lutein		30.8	23.3	21.3	12.1	8.8	6.5	9.1
Zeaxanthin		3.6	8.8	10.0	7.9	7.5	7.3	11.2
Mutatoxanthin		—	1.1	1.0	1.5	—	0.7	—
Isolutein		2.4	2.2	1.8	—	0.4	—	1.0
trans-Antheraxanthin		5.1	4.6	6.1	7.9	8.9	6.8	8.1
cis-Antheraxanthin		—	1.6	2.1	2.5	3.9	4.1	2.2
Luteoxanthin		2.0	6.0	3.7	4.2	—	2.4	—
trans-Violaxanthin		6.3	5.7	4.6	5.7	6.5	2.4	5.1
cis-Violaxanthin		9.1	6.2	7.5	7.9	8.8	4.2	4.4
Neoxanthin		15.4	13.8	11.8	10.7	9.2	8.8	5.2

biosynthetic steps, namely phytofluene and ζ-carotene, as well as γ-carotene, the immediate precursor of β-carotene, were detectable.

The carotenoid changes occurring at the last ripening stages were also followed in detached fruits. Three ripening stages were selected: harvest-ripe, intermediate and fully ripe. The results are shown in Table 2.

The detached fruits had a higher carotenoid content at harvest (128 μg/g). However, as characterized by the cryptoxanthin level, their ripening stage was less advanced (equivalent to stage V in Table 1). Unlike tree-ripened fruit, they contained small amounts of lycopene.

In these fruits, cryptoxanthin reached its highest level at the intermediate stage, representing 50% of total carotenoids. In the fully ripe fruits with a very high carotenoid content of 491 μg/g, the same changes in carotenoid pattern occurred as in the tree-ripened fruits; lycopene was again present as the second major pigment, however at a lower level (8.2%).

The overall carotenoid pattern was similar in both assays and the absolute amounts of the major pigments increased during ripening.

DISCUSSION

The carotenoid pattern of the 'Triumph' persimmon does not differ from the three Japanese varieties analysed in detail earlier [6]. During ripening, the changes of the total carotenoid content showed a minimum when the fruit began to turn orange and then increased gradually as chlorophyll disappeared. At the last stages the carotenoids were massively synthesized.

Such a minimum in the ripening pattern, i.e. the changes in the total carotenoid level during development, has been found to occur in many fruits in which ripening involves the transformation of chloroplasts into chromoplasts [7–10, 12]. The minimum corresponds to the grana disorganization that precedes the *de novo* carotenogenesis in the developing chromoplasts [13].

The carotenoid pattern changes in the persimmon peels paralleled these transformations, the chloroplast–carotenoid pattern changing gradually into a chromoplast pattern. The major chromoplast carotenoids characterizing the harvest fruit were predominantly cryptoxanthin accompanied by lower levels of zeaxanthin, antheraxanthin and violaxanthin.

The constant increase of the cryptoxanthin level makes it possible to use cryptoxanthin as an index of maturity as was done here. Similarly, the variation of the violaxanthin content in the peel of 'Golden Delicious' apple, which increases in parallel with the ripening process, may be used as an index of maturity [14].

An enhanced carotenogenesis occurred after harvest, with cryptoxanthin at the highest level of 50%, and a total carotenoid content of almost 500 μg/g, which was not attained in the tree-ripened fruits.

Likewise in tree-ripened 'Golden Delicious' apple, the maximum violaxanthin level was 40%, whereas in detached fruits it attained 58% and the total carotenoid content almost doubled [7].

For apples grown in Germany, temperature affects the difference in carotenogenesis in fruits ripened on and off the tree. For persimmons grown in Israel, since there are no drastic temperature changes, the factors responsible

Table 2. Carotenoid changes in the peel of persimmon (*Diospyros kaki*) cv Triumph during post-harvest ripening

	Ripening stages		
	Harvest-ripe	Intermediate	Fully ripe
Total carotenoids (μg/g fr. wt)	128.0	366.0	491.0
Carotenoid pattern (% of total carotenoids)			
Phytofluene	—	—	0.4
α-Carotene	1.6	1.2	1.0
β-Carotene	9.4	7.6	6.7
Mutatochrome	—	0.7	—
γ-Carotene	—	0.4	—
Lycopene	1.1	0.5	8.2
β-Cryptoxanthin	29.2	50.0	48.2
Cryptoxanthin 5,6-epoxide	0.9	1.2	1.9
Cryptoflavin	0.7	2.1	2.9
Lutein	12.4	5.5	4.1
Zeaxanthin	9.3	9.7	5.9
Mutatoxanthin	0.8	4.7	1.8
Isolutein	0.5	—	0.3
trans-Antheraxanthin	5.4	2.0	4.8
cis-Antheraxanthin	6.2	2.2	2.3
Luteoxanthin	1.7	1.8	1.9
trans-Violaxanthin	6.9	3.7	3.8
cis-Violaxanthin	6.7	1.5	2.0
Neoxanthin	7.2	5.2	3.8

for the enhanced carotenogenesis are not known.

With the completion of the ripening, the carotenoid pattern changed since lycopene appeared as an additional major pigment. This development is very peculiar and so far has not been encountered in other fruits. Generally, the biosynthesis of the chromoplast pigments begins with the formation of these plastids.

Also uncommon is the difference in the lycopene content between tree-ripened and detached fruits, 22 and 8% in the same cultivar both at the same ripening stage. This would explain the difficulty encountered in the classification of the 40 varieties of Japanese persimmon into well-defined groups, according to their lycopene content, since many varieties showed high variability [6].

A possible explanation for the unusual carotenoid pattern change may be that the turnover rate slows down at the final ripening stage. As a consequence, early precursors accumulate. This phenomenon is usually encountered in ripening fruits in which phytofluene and ζ-carotene are the accumulating precursors and not lycopene. There is also a precursor–product relationship between cryptoxanthin and lycopene.

Two other, less probable, explanations may also be considered. Lycopene synthesis may be delayed in a certain tomato mutant, the pigment being suddenly produced at muturation within 24–30 hr [15].

Another possibility is that there are two biosynthetic pathways, one for cryptoxanthin and another for lycopene, the latter being active at advanced maturation. Such separate pathways for β-carotene and lycopene are known to occur in tomato [16–18].

EXPERIMENTAL

Fruits were provided by the Department of Fruit and Vegetable Storage, Agricultural Research Organization, Beit Dagan, Israel.

For the first assay, fruits were picked at 2 week intervals. At stage VI the fruits were harvested. After harvest some fruits were left on the tree for ripening and analysed when ripe.

For the second assay, the post-harvest ripening and the classification into maturity categories according to ripening parameters such as external colour and firmness were carried out by the Department of Fruit and Vegetable Storage.

The analytical methods were as previously reported [10]. The pigments were separated by successive TLC on two different adsorbents according to the method of Gross [19].

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