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A COMPARATIVE STUDY OF THE CAROTENOID PIGMENTS IN JUICE OF SHAMOUTI, VALENCIA AND WASHINGTON ORANGES, THREE VARIETIES OF *CITRUS SINENSIS*

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Abstract—The carotenoid composition of three varieties of *Citrus sinensis* grown in Israel was investigated. The pigment pattern was similar in all three varieties although some quantitative variation was observed. The differences in color between Valencia, Shamouti and Washington Navel varieties result from these quantitative differences in carotenoid composition The ratio of the orange pigments (λ_{max} 445 nm) to the yellow pigments (420 nm) decreased from Valencia through Shamouti to Washington Carotenoids previously not reported in *Citrus* such as γ-carotene, rubixanthin, sintaxanthin and its OH-derivative and lutein 5,6-monoepoxide, were identified Two new carbonyl pigments with unknown structure were also detected

INTRODUCTION

IN THE present study, the carotenoids of orange juice were investigated by means of column chromatography combined with TLC This enabled us to separate more than fifty pigments in Shamouti orange juice, including some heretofore unknown in this variety [1] The carotenoid pigments of Valencia and Washington Navel, the other two principal orange varieties grown in Israel, were also investigated using the same techniques

The object of this investigation was to determine whether color differences between the different juices derive from either the variation in type or the amount of the different carotenoids In addition, knowledge of the distribution of the carotenoids in the fruit, may prove useful in taxonomic studies The chemotaxonomic significance of the carotenoids is well established for lower plants such as bacteria, algae and fungi [2–5] In higher plants, the pigments of the fruit and flower could well be of importance in determining phylogenetic relationships However, the carotenoids of higher plants have not as yet been sufficiently studied The methods employed in this work enable even minor carotenoids to be detected and eventually these could be used as characteristic indicators

RESULTS

In Table 1 the carotenoid distribution of the three varieties investigated is given The pigments are presented in order of increasing adsorption affinity on the several adsorbents used for column and TLC A similar pattern was found in the three sweet orange varieties However, some differences could be observed These consist in the fact that not all the pigments, or different isomeric forms of the same pigment, are common to all three varieties, as may be seen from Table 1

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[4] H KLEINIG, *J Phycol* **5**, 281 (1969)
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TABLE 1 CAROTENOID DISTRIBUTION IN THE JUICE OF THREE VARIETIES OF *Citrus sinensis*

Carotenoid	Varieties Shamouti	 Valencia	 Washington Navel
Fraction I-Hydrocarbons			
Phytoene	+	+	+
Phytofluene a	+	+	+
Phytofluene b	−	+	+
α-Carotene	+	+	+
β-Carotene	+	+	+
ζ-Carotene	+	+	+
Neo-β-carotene	+	−	−
ζ-Carotene-like	+	−	−
γ-Carotene	+	+	−
Intermediate			
Mutatochrome	+	+	+
β-Apo-10′-carotenal	+	+	+
Fraction II A-Monols			
cis-OH—α-carotene	−	+	−
OH—α-carotene, 5,6-epoxide	+	−	−
OH—α-carotene	+	+	+
OH—α-carotene-like	+	+	+
Poly-*cis*-Cryptoxanthin a	+	+	−
Poly-*cis*-Cryptoxanthin b	+	+	−
Fraction II B-Monols			
Rubixanthin	+	+	+
OH—α-carotene 5,8-furanoxide	+	−	−
Sintaxanthin	+	+	+
cis-Cryptoxanthin	+	+	+
Cryptoxanthin a	+	+	+
Cryptoxanthin b	+	+	+
Cryptoxanthin-like a	+	+	−
Cryptoxanthin-like b	+	+	−
cis-Cryptoxanthin-like	+	−	−
Fraction III A-Diols			
Cryptoflavin	+	+	+
cis-Cryptoflaivn	−	+	+
Cryptoxanthin 5,6-epoxide-5′,8′-furanoxide	+	−	+
Cryptoxanthin 5,8,5′,8′-difuranoxide	−	+	−
Unknown 418	+	−	−
Isolutein-like	−	+	−
Lutein-like	+	+	+
Lutein	+	+	+
cis-Lutein	+	+	+
Isolutein	+	+	+
cis-Isolutein	+	+	+
Violaxanthin	+	+	+
cis-Violaxanthin a	−	+	−
cis-Violaxanthin b	−	+	−
cis-Violaxanthin c	−	+	−

TABLE 1.—*Continued*

Carotenoid	Shamouti	Varieties Valencia	Washington Navel
Fraction III B-Diols			
Unknown 455	+	+	−
Chrysanthemaxanthin	+	−	−
Unknown 420	+	−	−
Citraurin	+	+	+
OH—Sintaxanthin a	+	+	+
OH—Sintaxanthin b	+	+	+
OH—Sintaxanthin 5,6-epoxide	−	+	−
Zeaxanthin	+	+	+
Antheraxanthin	+	+	+
cis-Antheraxanthin	+	−	−
Luteoxanthin a	+	+	+
Luteoxanthin b	+	+	+
Fraction III C-Polyols			
Carbonyl 408	+	+	+
Carbonyl 426 a	+	+	+
Carbonyl 424 b	−	+	−
Mutatoxanthin	+	+	+
cis-Mutatoxanthin	+	+	−
Auroxanthin a	+	+	+
Luteoxanthin-like	+	+	+
Trollichrome a	+	+	+
Auroxanthin b	−	+	−
Sinensiaxanthin	−	+	−
Trollichrome b	+	+	+
Trollixanthin a	+	+	−
Neoxanthin a	+	+	+
Trollixanthin b	+	+	+
Neoxanthin b	+	+	+

Pigments which have not been previously reported in citrus, as well as some unidentified pigments are presented in Table 2. The carbonyl pigments 408, 426a and 424b were present only in minute amounts, so that determination of their structure was impossible. The quantitative distribution of the three varieties is given in Table 3.

DISCUSSION

The total carotenoid content was about 6–10 μg/ml of juice in Shamouti, 10–15 μg/ml in Valencia and 4–7 μg/ml in Washington. In all three varieties the quantitative distribution was similar. The complex diol-polyol fraction is predominant, accounting for about 70% of the total carotenoids.

Some quantitative differences should be emphasized. In Valencia, the most highly colored juice, there was twice as much of the colored hydrocarbon fraction as in the other two varieties. Within this fraction the proportion of the three pigments, α, β, and ζ-carotene are also different. In Valencia there was also twice as much OH—α-carotene as in the other two

TABLE 2 CHARACTERIZATION OF SOME LESS COMMON CAROTENOIDS DETECTED IN THE JUICE OF THREE VARIETIES OF *Citrus sinensis*

Identification	Abs max* (nm)	Epoxide test (HCl treatment) Colour	Hypsochromic Shift (nm)	Carbonyl test Abs max after reduction with $NaBH_4$
Phytofluene b	328, 348, 367	—	—	—
Neo-β-carotene	422, 444, 470	—	—	—
ζ-Carotene-like	378, 402, 424	—	—	—
β-Apo-10′-carotenal	435	—	—	390, 410, 432
	427 (Washington)	—	—	370, 397, 417
Sintaxanthin	447	—	—	395, 421, 450
Unknown 418	397, 428, 445	—	—	—
Isolutein-like	412, 440, 470	green	20	—
Isolutein	416, 440, 470	green	18	—
cis-Isolutein	335, 415, 438, 468	green	15	—
cis-Violaxanthin a	325, 408, 430, 458	green–blue	32	—
cis-Violaxanthin b	322, 410, 432, 460	green–blue	34	—
cis-Violaxanthin c	323, 410, 435, 465	green–blue	34	—
Unknown 456†	430, 456, 488	—	—	—
Unknown 420†	395, 420, 440	green	15	—
OH—Sintaxanthin a	447	—	—	400, 423, 446
OH—Sintaxanthin b	444	—	—	400, 420, 445
OH—Sintaxanthin-5,6-epoxide‡	445	blue	15	388, 412, 438
Carbonyl 408	408	—	—	334, 356, 377
Carbonyl 426 a†	426	—	—	373, 395, 418
Carbonyl 424 b†	424	—	—	370, 393, 417
Sinensiaxanthin‡	370, 390, 413	blue	20	—

* Phytofluene determined in hexane, neo-β-carotene, ζ-Carotene-like, β-Apo-10′-carotenal and sintaxanthin in petroleum ether, and the others in ethanol

† New unidentified pigments

‡ Tentative identification

varieties In Shamouti the ratio OH—α-carotene fraction/cryptoxanthin is about 1 10, while in Valencia it is about 1 3 Cryptoxanthin was present in Shamouti in slightly greater quantities than in Valencia or Washington Of more interest is lutein, in Valencia there was about twice as much as in Shamouti while in Washington, lutein was present in even larger amounts The very high percentage of zeaxanthin present in Valencia is also worthy of note, as is the high level of antheraxanthin In Washington, the relatively high level of auroxanthin is unusual Our data agree well with that of Goodwin [6]

Of genetic interest is the fact that unique carotenoids such as sintaxanthin and OH-sintaxanthin, which appear in significant amounts only in hybrids,[7] could be detected as minor components in all three *Citrus* varieties Rubixanthin, which is considered a unique pigment of the *Rosa* species,[8] also appeared as a minor pigment in *Citrus*

Generally it appears that the Valencia variety, which is quantitatively the richest juice in carotenoids, has the most complex pigment pattern The Washington variety, with the lightest

[6] T W GOODWIN, in *The Biochemistry of Fruits and their Products* (edited by A C HULME), Vol I, p 305, Academic Press, New York (1970)

[7] H YOKOYAMA and M J WHITE, *Phytochem* **5**, 1159 (1967)

[8] N ARPIN and S LIAAEN-JENSEN, *Phytochem* **8**, 185 (1969)

TABLE 3 QUANTITATIVE CAROTENOID DISTRIBUTION IN THE JUICE OF THREE VARIETIES OF *Citrus sinensis*

Pigment	Per cent of total carotenoids*		
	Shamouti	Valencia	Washington Navel
Phytoene†	5 70	4 20	5 60
Phytofluene†	2 40	2 00	2 60
Fraction I total	3 00	6 00	3 30
α-Carotene	0 36	1 00	0 70
β-Carotene	1 24	2 00	0 80
ζ-Carotene†	1 40	3 00	1 80
Intermediate and Fraction II A total	1 60	3 60	1 80
Mutatochrome	0 02	traces	traces
β-Apo-10′-carotenal	0 03	0 10	traces
OH—α-Carotene	1 05	2 90	1 80
Poly-*cis*-Cryptoxanthin	0 50	0 60	—
Fraction I B total	15 00	10 60	12 50
Rubixanthin	0 05	traces	traces
Sintaxanthin	0 07	traces	traces
cis-Cryptoxanthin	2 00	0 90	1 50
Cryptoxanthin	12 88	9 70	11 00
Fraction III A total	13 00	13 20	18 30
Cryptoflavin	0 70	0 30	0 50
Cryptoxanthin diepoxide	0 10	0 05	0 20
Lutein	5 20	9 00	11 20
Isolutein	4 00	2 00	4 70
Violaxanthin	3 00	1 85	1 70
Fraction III B total	20 00	27 80	19 20
Chrysanthemaxanthin	0 07	—	—
Citraurin	0 11	traces	traces
OH—Sintaxanthin	2 48	1 90	1 30
Zeaxanthin	6 20	10 20	4 75
Antheraxanthin	6 83	9 30	6 15
Luteoxanthin	3 98	6 40	7 00
Fraction III C total	39 30	32 60	36 70
Carbonyl 408	0 39	traces	traces
Carbonyl 426	0 78	0 50	0 75
Mutatoxanthin	15 13	14 00	13 00
Auroxanthin	0 58	1 20	4 30
Luteoxanthin-like	6 92	5 10	3 90
Trollichrome	1 46	2 80	3 50
Trollixanthin	9 64	6 50	9 10
Neoxanthin	3 97	2 50	2 16

* Expressed as β-carotene Isomeric forms of the same pigments were determined together. Minor pigments were not recorded (traces > 0 02)

† Phytoene, phytofluene and ζ-carotene were read at the wavelengths of maximal absorption using their reported extinction coefficients

color, is the least complex. The reddest tinted variety, Valencia, did not contain any red pigment which was not also present in Washington Such qualitative differences can also be found, for example, in varieties of pepper (*Capsicum annuum*) The red variety is characterized by the presence of capsanthin and capsorubin which are absent in the yellow variety, which contains, instead, much lutein [9] The same is true for different tomato strains, where not only do the quantities of lycopene differ being totally absent from the tangerine variety, but β-carotene predominates in the High Beta strain [10,11]

A similar situation is found among varieties of maize where differences in color can be ascribed to differences in the concentration of zeaxanthin [12] The same is true for carrot varieties, where the most highly colored was the richest in β-carotene [13,14]

The differences in color among the three varieties of orange studied is not the result of major qualitative differences in the complex carotenoid content of the juice Rather, the color is due to (a) the total carotenoid concentration, and (b) the ratio of orange pigments that absorb towards longer wavelengths (445 nm) to yellow pigments that absorb at some shorter wavelengths (420 nm) The ratio of orange to yellow pigments decreases in the order of Valencia, Shamouti, Washington, which corresponds to the order of decreasing color of the juice. A comparison of our results with these obtained by Curl[15,16] is of interest. The differences found between the Californian varieties of Valencia and Washington Navel analyzed by him and the two varieties grown in Israel may result both from the differences in analytical methods and the effects of environmental factors We could not find such specific pigments as valenciaxanthin, valenciachrome and trolliflor. In Washington, we found only 1 7% violaxanthin versus 45% reported in the American variety. The highest percentage of violaxanthin we detected was 12% in Shamouti juice at the beginning of the season In addition the quantity of ζ-carotene in Washington Navel grown in Israel was substantially less than that grown in California. It would be of interest therefore, to study the effect of environmental conditions upon the color and carotenoic composition of *Citrus*.

EXPERIMENTAL

Juice The juice used in this study was obtained either by hand pressing of commercially available oranges or collected from the pressing machines of citrus processing factories The juice was obtained during the mid and late season, when the fruit is in its highly pigmented state All the operations were carried out in subdued light The juice was examined immediately or stored at −18° until used

Methods The analytical techniques and methods have been described previously [1]

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Key Word Index—Citrus sinensis, Rutaceae, orange varieties, carotenoids.