The Carotenoids of Tangerines

A. LAURENCE CURL and GLEN F. BAILEY

Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, Calif.

The carotenoids in tangerine peel and pulp (fruit flesh) have been fractionated by countercurrent distribution, followed by chromatography. Factors contributing to the redder color of the tangerine, as compared to the orange, were much higher concentrations of cryptoxanthin (pulp and peel), β-carotene (pulp), and a hydroxy-canthaxanthinlike substance (peel). Minor constituents not previously reported from oranges included lycopene, γ-carotenelike and hydroxycanthaxanthinlike fractions from the pulp, and lycopene and a rubixanthinlike fraction from the peel. A minor phytofluenelike band was found on chromatography on the column between α-carotene and β-carotene, in both pulp and peel carotenoids.

VERY LITTLE has been published on the carotenoids of tangerines (*Citrus reticulata* Blanco) grown in the United States. Matlack in 1928 (*8*) reported that the pigments of the tangerine were apparently carotin and xanthophyll. Zechmeister and Tuzson (*17*) investigated the carotenoids of Italian mandarin oranges, both peel and fruit flesh, and reported that in both cases a complicated mixture was present: β-Carotene, cryptoxanthin, and lutein were isolated. The tangerine, both peel and pulp (fruit flesh), is distinctly redder in appearance than the principal varieties of oranges grown in the United States. This was confirmed by studies with the Hunter color difference meter by Huggart and Wenzel (*5*). A comparison was therefore made of the carotenoids of tangerines (both pulp and peel) with those of oranges to see whether the difference in color is caused by a higher content of carotenoids, or whether the increased redness is due to pigments not present in oranges or to greater concentrations of some of the redder pigments which are present in oranges. Countercurrent distribution was used to separate the pigments into fractions having similar distribution coefficients, followed by chromatography to separate the fractions into the individual components, in a manner similar to that used in earlier work on oranges (*1–4*).

Experimental

Fruit purchased at a local market in January 1955 was handpeeled; the peeled fruit (pulp) amounted to 76% of the total weight. The peels and pulp were worked up separately. The peels were extracted by the method previously described for aged orange peels (*4*) and the carotenoid extract was saponified (*1*).

The pulp was blended with an equal volume of water in the presence of an excess of calcium carbonate and the suspension was mixed with an equal volume of methanol. After standing overnight at 1° C., the mixture, with filter aid added, was filtered on a Büchner funnel, which was precoated with filter aid. After washing with a methanol-water mixture (1 to 1 by volume), the filter cake was extracted with acetone on sintered-glass funnels and the acetone extract was worked up as previously described, including saponification (*1*).

The color in the peel and pulp extracts, after saponification, was measured in an Evelyn colorimeter, using a 440-mμ filter and calculated as β-carotene. On this basis, the peel and pulp contained 186 and 26.5 mg. per kg., respectively. It was calculated that 69% of the carotenoids of the entire fruit were present in the peel. For comparison, in a sample of California Valencia oranges (*4*), the peel and pulp were found to contain 98 and 24 mg. per kg., respectively, with 62% of the total carotenoids in the peel.

Three countercurrent distribution runs were carried out in an all-glass Craig apparatus (*1*) with separate portions of saponified material (the unsaponifiable fraction). The system petroleum ether–99% methanol (1.8 to 1 by volume), with 97 transfers, was used to separate the pigments into hydrocarbons, monols, and diols plus polyols. In the case of the peel carotenoids, a second run was made with 197 transfers to separate, more completely, a fourth fraction, apparently monoether monols (Figure 1). The system consisting of benzene, petroleum ether, and 87% methanol (1:1:1.15 by volume), with 197 transfers, was used in the third run to separate the carotenoids into five fractions: hydrocarbons

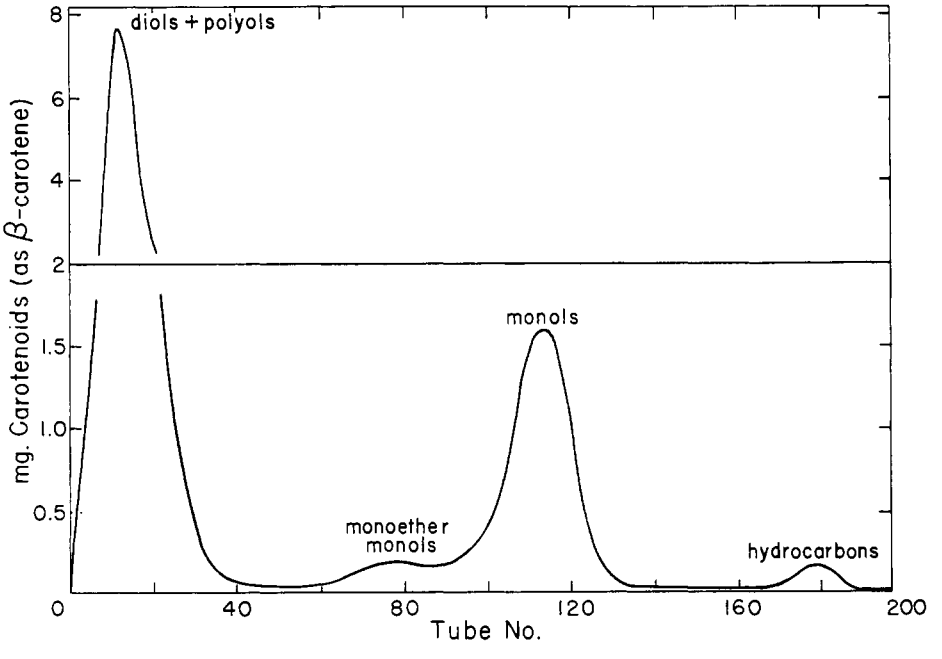

Figure 1. Countercurrent distribution of saponified carotenoids from tangerine peel

System petroleum ether–99% methanol

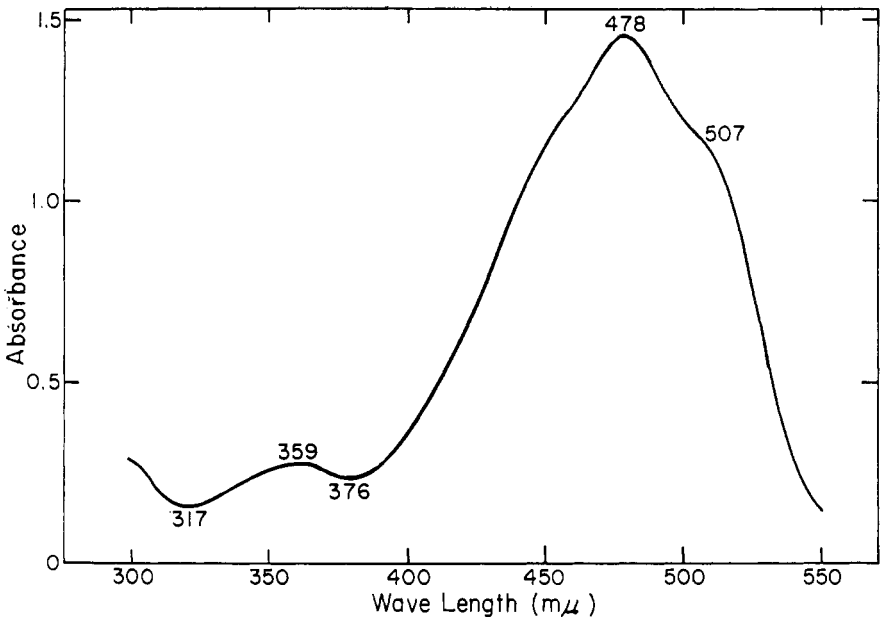

Figure 2. Spectral absorption curve of hydroxycanthaxanthinlike pigment from tangerine peel, in benzene

Values for absorbance are relative

plus monols, diols, monoether diols, diether diols, and polyols. The fractions (hydrocarbon and monol from the first run; diol, monoether diol, diether diol, and polyol from the third run) were chromatographed on columns of magnesia (Westvaco No. 2642) plus filter aid (1 to 1 by volume); solutions of acetone or ethyl alcohol in petroleum ether or benzene were used as eluants. Spectral absorption curves were run in a Cary recording spectrophotometer.

Results and Discussion

The results of the countercurrent distribution runs with both tangerine pulp and peels are summarized in Table I; for comparison, data previously obtained for orange juice and peel are included. The N_{100} values (tube number of the maxima calculated on the basis of 100 transfers) of the tangerine pulp and peel fractions were in good agreement and also checked well with those obtained for orange; hence these values are omitted from Table I.

The most outstanding feature of the tangerine carotenoids (Table I) was that they had a much greater proportion of monols than did the orange carotenoids (*4*). In the tangerine pulp carotenoids, the hydrocarbon fraction was also rather high and the polyol fraction was low. A monoether monol fraction (Figure 1) was present in tangerine peel carotenoids as in orange peel carotenoids, but it was not observed in the tangerine pulp carotenoids. The diether diol fraction was unusually high in the tangerine peel carotenoids, though not as high as previously found in orange peel carotenoids (*4*).

The constituents obtained on chromatography are given in Table II by fractions, the constituents obtained from each fraction being listed in the order of elution, the top being the first eluted. Some bands were accompanied by smaller adjacent bands with absorption maxima several millimicrons lower, which previous experience had shown to be cis isomers. These are not listed separately in Table II but are combined with the all-trans isomers. Spectral absorption maxima were omitted from Table II because most of these values were very similar to those previously obtained for orange carotenoids (*1–4*); the spectral absorption maxima of several constituents not previously found in oranges are given in Table III. The percentages of total carotenoids given in Table II are based on the total absorption of each constituent at its principal maximum; as the values for the specific absorption coefficients are not known for most of the constituents, it was assumed that these were all the same. This is not strictly true, but most of the known values lie in the same general range, so that approximate values for percentages of the total carotenoids may be calculated by this means.

Table I. Countercurrent Distribution of Saponified Carotenoids from Tangerine Pulp and Peel in Comparison with Carotenoids from Oranges

(Measured as β-carotene)

	Approximate % of Total Carotenoids			
	Tangerines		Oranges	
Fraction	Pulp	Peel	Juice (*1*)	Peel (*4*)
Hydrocarbons	9	2	6	3
Monols	43	27	13	7
Monoether monols	. .	3	. .	3
Diols	9	13	22	5
Monoether diols	16	12	26	12
Diether diols	21	37	23	66
Polyols	3	5	10	5

Hydrocarbon Fraction. In the pulp carotenoids, the ratio of β-carotene to α-carotene (14 to 1) was much greater than that found for the peel carotenoids, or previously for carotenoids from oranges, where ratios of 2 or 3 to 1 are usual. This probably contributes to the redness of tangerines to some exent, as β-carotene is considerably redder than α-carotene. Phytoene, phytofluene, and ζ-carotene were all major constituents of the hydrocarbon fraction, as they are in orange carotenoids.

In both the pulp and peel carotenoids, a minor band with greenish fluorescence in ultraviolet light was observed on the column between β-carotene and α-carotene. The spectral absorption maxima (Table III) of these bands were very close to those of phytofluene. This fraction appears to be identical with or very similar to the colorless polyene A obtained from tomatoes by Trombly and Porter (*14*).

A minor fraction obtained from tangerine pulp had spectral absorption maxima at a considerably higher wave length than for β-carotene, but somewhat lower than those reported for γ-carotene (Table III). It did not appear to be a pure all-trans compound, and there was not sufficient material available for further investigation. A very minor fraction was identified as lycopene by its spectral absorption curve (Table III) and behavior on countercurrent distribution and chromatography. Lycopene has not been found in oranges.

Monol Fraction. The most striking feature of the monol-fraction carotenoids was the high percentage of cryptoxanthin from both peel and pulp. The values were much higher than those found in oranges (*1*, *4*). Because cryptoxanthin is redder than any of the other principal carotenoids of tangerines and oranges, except β-carotene and zeaxanthin, it is very probable that the high cryptoxanthin content is mainly responsible for the redder color of tangerines as compared to the color of oranges. The ratio of cryptoxanthin to hydroxy-α-carotenelike was much greater than has been found in oranges. In the pulp, small amounts of fractions identified as cryptoxanthin epoxide (absorption maxima in petroleum ether, 474, 445, and 423 mμ) and cryptoflavin (the corresponding furanoxide) were found; these have been found in some orange juice carotenoid preparations, but not in all.

Table II. Carotenoid Constituents Obtained from Tangerine Pulp and Peel

Fraction	Constituent	Approximate % of Total Carotenoids: Pulp	Peel
Hydrocarbon			
	Phytoene	5.8	4.2
	Phytofluene	7.2	3.5
	α-Carotene	0.3	0.2
	Phytofluenelike	0.1	0.1
	β-Carotene	4.1	0.4
	ζ-Carotene	6.9	2.0
	γ-Carotenelike	0.1	...
	Lycopene	0.1	0.02
Monol			
	Hydroxy-α-carotenelike	1.0	0.6
	Cryptoxanthin epoxide	0.9	1.4
	Cryptoxanthin	33	24
	Hydroxy-α-carotene furanoxidelike	...	0.4
	Cryptoflavinlike	0.8	3.4
	Rubixanthinlike	...	0.2
	Cryptochromelike	...	0.1
Diol			
	Lutein	2.9	3.3
	Zeaxanthin	3.3	3.5
	Hydroxy-canthaxanthinlike	0.1	2.7
Monoether diol			
	Antheraxanthin	9.7	6.2
	Mutatoxanthins	2.2	2.8
Diether diol			
	Violaxanthin	14	24
	Luteoxanthins	3.5	9.1
	Auroxanthins	0.4	1.9
Polyol			
	Valenciaxanthin	0.2	0.4
	Sinensiaxanthin	0.2	1.1
	Trollixanthinlike	1.0	2.6
	Trollein	0.9	...
	Trollichromelike	0.3	0.7

In the peel, the monol fraction was considerably more complicated than in the pulp, as was also the case with orange peel (*4*). The cryptoflavinlike fraction was unusually large; two bands which appeared to be all-trans cryptoflavins were found, as was the case with most furanoxides previously examined. A minor band had absorption maxima (445, 419, and 397 mμ in petroleum ether) corresponding to a *cis*-hydroxy-α-carotene furanoxide; this band had considerably greater fine structure in the spectral absorption curve than the cryptoflavinlike substance. A very minor band, highest on the column, had absorption maxima (424, 400, and 379 mμ in petroleum ether) near those of auroxanthin (zeaxanthin difuranoxide) and appeared to be cryptochrome (cryptoxanthin difuranoxide). Bands similar to the latter two were previously isolated from orange peel carotenoids (*4*), but neither was present in sufficient quantity for further study. Another minor band (Table III) had spectral absorption maxima corresponding to rubixanthin (hydroxy-γ-carotene) (*6*), though it could also have been gazaniaxanthin (*16*), the structure of which has not yet been fully elucidated. Both of these have the same chromophoric system as γ-carotene.

Diol Fraction. This fraction, as in orange juice carotenoids, was found to consist mainly of zeaxanthin and lutein. The peel also contained a fairly large amount—the pulp a trace—of a third component which had been found previously in orange peel (*4*). The spectral absorption curve (Figure 2) was changed only slightly by iodine isomerization which ruled out its being a poly-cis compound, such as prolycopene or pro-γ-carotene (*15*). The shape of the spectral absorption curve suggested that it might be a ketonic carotenoid such as capsanthin (*12*) or canthaxanthin (*13*), the principal absorption maxima of which are 484 and 480 mμ in benzene,

Table III. Spectral Absorption Maxima of Some Carotenoid Fractions from Tangerines Not Found in Oranges

Constituent	Solvent	Maxima, Mμ
Phytofluene-like[a]	Pet. ether	368, 348, 333
γ-Carotene-like[b]	Pet. ether	488, 459, 434
Lycopene[b]	Pet. ether	503, 471, 444
Rubixanthinlike[a]	Pet. ether	492, 461, 434, 353

[a] Peel. [b] Pulp.

respectively. The latter is in better agreement with values obtained for this fraction from orange and tangerine peels (478 to 79 mμ).

Canthaxanthin was recently shown to be identical with 4,4′-diketo-β-carotene (*10*) which was prepared synthetically by Petracek and Zechmeister (*11*). The reported behavior of canthaxanthin on chromatography and partition between hexane and 95% methanol does not match that of the fraction obtained in the present work. The partition of 4,4′-diketo-β-carotene was reported as 50 to 50, whereas that of 3,3′-dihydroxy-β-carotene (zeaxanthin) was 11 to 89 (*9*), corresponding to distribution coefficients of 1.0 and 8.1, respectively. The fraction obtained in the present work was found in the same countercurrent distribution fraction as zeaxanthin and on chromatography occurred well above it on the column; it may be 3-hydroxy-4,4′-diketo-β-carotene, which is apparently unknown. Lutein was eluted from the column by 3.5% ethyl alcohol in petroleum ether, zeaxanthin by 5 to 7%, and the canthaxanthinlike fraction by 10 to 15%. However, 3,3′-dihydroxy-4,4′-diketo-β-carotene is a known carotenoid, astaxanthin (*7*). In view of the unusual occurrence in orange and tangerine peels of a number of monols apparently containing one or two cyclic ether groups, the presence of a monol diketone would not be surprising.

Monoether Diol Fraction. The components of this fraction resembled closely those reported from orange juice (*3*): antheraxanthin, *cis*-antheraxanthin, and mutatoxanthins a and b. In the case of the pulp carotenoids only, a *cis*-mutatoxanthin was found on the column between mutatoxanthins a and b.

Diether Diol Fraction. The components of this fraction also resembled closely those reported from orange juice (*3*): violaxanthin, *cis*-violaxanthin, one all-trans luteoxanthin band, two *cis*-luteoxanthin bands, and auroxanthins. In the case of the peel carotenoids, three auroxanthin bands were found, one a cis isomer, the others all-trans; in the pulp carotenoids, the amount of auroxanthins was small and was all eluted as one subfraction. Violaxanthin was much greater in amount than any of the other diols, in the peel carotenoids, and was approximately equal to the cryptoxanthin.

Polyol Fraction. This fraction was small in the pulp carotenoids and somewhat larger in the peel carotenoids, as compared with the corresponding fractions from oranges. Valenciaxanthin, sinensiaxanthin, and the trollixanthinlike substance were found as in orange juice xanthophylls (*3*); of the corresponding furanoxides only the trollichromelike fraction was observed. In the case of the pulp carotenoids, trollein (*2*), a nonether polyol, was found; this

carotenoid is difficult to separate chromatographically from the trollixanthin-like substance, which usually is found both as all-trans and as cis isomers, hence may sometimes be overlooked in the presence of somewhat greater quantities of the trollixanthinlike substances.

Nearly all subfractions obtained on chromatographing the diether diol peel fraction, three from the polyol peel fraction, and one from the monoether diol peel fraction, had very high single peaks on the spectrophotometer curves in the region of 300 to 325 mμ (in benzene). In the diether diol fractions, these peaks were successively at 314, 325, 314, and 303 mμ; in polyols at 300, 319, and 324 mμ; and in the monoether diol at 313 mμ. Similar peaks did not occur in the corresponding pulp fractions, but they had been found previously in fractions obtained from orange peel (*4*). These peaks may be caused by some of the less-volatile, peel-oil constituents, containing three or four conjugated double bonds.

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NUTRIENTS IN FROZEN FOODS

Amino Acids in Nine Frozen Vegetables

D. M. MURPHY, B. E. KLINE, R. N. ROBBINS, L. J. TEPLY, and P. H. DERSE

Wisconsin Alumni Research Foundation, Madison, Wis.

Ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) were determined on composites of 102 sets of samples, representing 1953 and 1954 production seasons for nine frozen vegetables. Sample packages were taken from commercial processing lines of freezing plants in all United States production areas. Samplings were performed at statistically predetermined intervals to provide representative samples.

TO SUPPLEMENT the limited data in the literature on nutrients in frozen foods, the National Association of Frozen Food Packers has undertaken the sponsorship of a comprehensive program in nutritional research. This report gives the results of the determination of ten amino acids in nine frozen vegetable products, which contain appreciable amounts of protein. Although arginine and histidine are not considered by all investigators to be essential for humans, they have been included in most previous work on fresh and prepared foods and, for this reason, were also included in this study. A previous paper from this laboratory by Burger and coworkers (*7*) reported the vitamin, mineral, and proximate composition of 51 frozen fruits, juices, and vegetable products, including the nine frozen vegetables used in this study.

Sampling and Sample Preparation

The statistical sampling plan employed has been described by Schmitt and Jessen (*6*). Packages were taken from commercial processing lines of freezing plants in all United States production areas. The samples were taken at statistically predetermined intervals to provide for representative sampling with regard to variables in weather, varieties, harvesting, processing, packaging, and grades. The coded sets were shipped under 0° F. refrigeration to a cold storage warehouse in Madison, Wis.

One hundred and two sets of samples were subjected to amino acid analysis. Each set was made up of an average of 38 packages of consumer-size frozen food packages. As described by Burger and associates (*7*), the sample sets were ground and thoroughly mixed, and assays on the frozen slurry were run as expeditiously as possible.

Assay Methods

Methods for proximate analysis have been described (*7*). The amino acids were determined by the microbiological method of Henderson and Snell (*2*). Tryptophan hydrolyzates were prepared by autoclaving the samples for 15 hours at 121° C., using 20 ml. of 5*N* sodium hydroxide per gram of protein. Com-

Table I. Proximate Composition of Frozen Vegetables

Frozen Food	No. of Sets	Solids, %	Ash, %	Ether Ext., %	Protein,[a] %	Carbohydrate, % Crude fiber	Carbohydrate, % Total (by difference)
Beans, baby lima	12	32.2	1.42	0.18	7.61	1.88	23.0
Beans, Fordhook lima	15	27.4	1.46	0.11	6.21	1.68	19.6
Broccoli spears	13	9.3	0.69	0.20	3.35	1.06	5.1
Brussels sprouts	11	11.5	0.84	0.15	3.28	1.19	7.2
Collard greens	3	11.0	1.04	0.38	3.27	1.01	6.3
Corn, cut	12	23.5	0.48	0.55	3.13	0.54	19.3
Peas, black-eyed	4	34.7	1.38	0.35	8.90	1.52	24.1
Peas, green sweet	26	19.0	0.72	0.31	5.30	1.83	12.7
Potatoes, French fried	6	36.8	1.06	6.08	2.63	0.56	27.0

[a] N × 6.25.