Part I – Comparison of Carotenoids of Valencia Orange Peel and Pulp

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A comparison of the peel and pulp of early-season Valencia oranges showed over 60% of the total carotenoids but less free diols and polyols in the peel. Qualitatively the components were nearly identical; quantitatively peel carotenoids contained much more violaxanthin. One constituent not previously found in late-season juices—apparently cryptoxanthin furanoxide—was found in both pulp and peel carotenoids; the corresponding epoxide was present in peel carotenoids. Countercurrent distribution studies of the carotenoids of aged canned Valencia orange juice, stored for 3 years at room temperature, showed no significant hydrolysis of xanthophyll esters or changes in composition of nonether carotenoids. The xanthophyll epoxides, about half of the total carotenoids of fresh orange juice, had entirely disappeared; the corresponding isomeric furanoxides were present in greater proportions than in fresh juice. Only auroxanthin was identified in the diether diol fraction. On chromatography trollein, valenciachrome, and a trollichromelike substance were obtained as well separated pairs of bands with practically identical spectral absorption curves. Trollein is apparently a nonether polyol.

THE CAROTENOIDS OF JUICE obtained from late-season (September to November) California Valencia oranges were investigated in previous studies at this laboratory (*1–3*). In the present work, a comparison was made with the carotenoids in fruit obtained in early May. Both peel and peeled fruit (pulp) were investigated, as in commercial extraction of orange juice some peel oil (which also contains carotenoids) is usually incorporated in the juice. The carotenoid mixtures of peel and pulp were qualitatively similar to those found previously in late-season juices. Quantitatively the pulp and peel carotenoids differed chiefly in that the latter contained a much higher percentage of violaxanthin. A constituent not previously found in juice was present in peel and to a lesser extent in pulp carotenoids, and was tentatively identified as cryptoxanthin furanoxide; the corresponding epoxide apparently occurred in the peel and probably in the pulp.

Experimental

Nine kilograms of California Valencia oranges was obtained at a local market in May 1953. The fruit was hand peeled; the peels and peeled fruit (pulp) amounted to 29 and 71% by weight, respectively. Subsequent operations were carried out in subdued light.

Extraction of Pulp. The peeled fruits (6066 grams) were cut in two and pressed in a ricer, yielding 2803 grams (46%) of juice, from which the carotenoids were extracted in the usual way (*1*). The residual pulp from the ricer was blended with acetone and filtered after standing overnight at 1° C. The filter cake was again extracted with acetone and filtered and the resulting filter cake was washed with acetone until no more color was extracted. The acetone extracts were worked up in the usual way (*1*). The carotenoid content of the combined three extracts was measured in an Evelyn photoelectric colorimeter using a 440-mμ filter, and calculated to be equivalent to 24 p.p.m. (as β-carotene) in the peeled fruit.

Extraction of Fresh Peels. One kilogram of the peels was extracted in a manner similar to that used for pulp, with initial pressing omitted. The carotenoid content was found to be equivalent to 98 p.p.m. of β-carotene in the peels. Calculated on a basis of 1 kg. of whole oranges, the carotenoids (as β-carotene) obtained from the peel and pulp amounted to 28 and 17 mg., respectively, or a total of 45 mg.

Extraction of Aged Peels. One kilogram of the peels was extracted after being stored in a closed container at 6° C. for 4 months. A more rapid process was used for the extraction of the aged peels, which were covered with absolute methanol and allowed to stand overnight at 6° C. The methanol solution was decanted off and discarded. The peels were blended with acetone and filtered, and the filter cake was exhaustively washed with acetone. The acetone extract was worked up as described (*1*). The carotenoids recovered amounted to 34.0 mg. (as β-carotene), a retention of 35% of that originally found.

Saponification. One half of the carotenoid solutions obtained from both pulp and fresh peel and all of that from aged peel were saponified (*1*). The recoveries of carotenoids in the saponified material were 92 and 74% for the fresh peel and pulp, respectively. In both cases a part of the color remained in the aqueous phase after dilution with water and extraction with ether; this color showed the presence of acidic pigments (*2*).

Countercurrent Distribution. Runs in a glass Craig apparatus were made on both unsaponified and saponified material. With unsaponified material from pulp and fresh peels, and saponified material from aged peels, 99 transfer runs were carried out with the system petroleum ether–99% methanol (1.8 to 1 by volume). With saponified material from pulp and fresh peels, 199 transfer runs were made with the system benzene–petroleum ether–87% methanol (1 to 1 to 1.15 by volume). The color in each (99 transfers) or every other (199 transfers) tube was measured and calculated as β-carotene. From these results the contents of the tubes for the saponified material were combined into five fractions each and evaporated in vacuo, and the residues were dissolved in benzene or petroleum ether. The unsaponified material was discarded.

Chromatography. The solutions in benzene or petroleum ether were absorbed on columns of magnesia (Westvaco No. 2642) plus filter aid (1 to 1 by volume), and fractionally eluted by solutions of acetone or ethyl alcohol in petroleum ether. The fractions obtained were evaporated in vacuo, the residue was dissolved in petroleum ether, ben-

zene, or ethyl alcohol, and spectral absorption curves were run in a Cary recording spectrophotometer.

Results and Discussion

Countercurrent Distribution of Unsaponified Carotenoids. The results, as summarized in Table I, showed separation into three major fractions for both pulp and peel carotenoids. In previous work (*1*) with saponified carotenoids it was shown that these fractions were composed of: I, hydrocarbons; II, monols; and III, diols and polyols. By analogy, with unsaponified material it is probable that the components of fractions I, II, and III contained 0, 1, and 2, or more free hydroxyl groups, respectively. The percentage of total carotenoids and the N_{100} value (Table I) were in good agreement for fraction I of the peel and pulp, but not for fractions II and III. The ratio of the percentages of fractions II and III for the peel carotenoids was 12 to 1, whereas for the pulp it was only 2.25 to 1, in good agreement with runs on late-season juices. Furthermore, the N_{100} values for fractions II and III in the peel and pulp samples were not in good agreement, indicating these fractions to be considerably different in composition. The results indicate a considerably higher percentage of free diols plus polyols in the pulp carotenoids.

Countercurrent Distribution and Chromatography of Saponified Pulp and Fresh Peel Carotenoids. The distribution of these carotenoids is shown in Figure 1. Earlier work (*1*, *3*) had shown that the five major fractions have the composition: I + II, hydrocarbons and monols; III A, diols; III B, monoether diols; III C, diether diols; and III D, polyols. The N_{100} values of these five fractions were in good agreement for the peel and pulp extracts, and agreed well with earlier results (*1*). The corresponding percentages of the several fractions in the peel and pulp carotenoids were different (Table II); fraction III C was much greater in the peel than in the pulp. However, the ratios of the percentages of the other four fractions for the peel and pulp carotenoids were in rather good agreement, indicating that, except for fraction III C, the composition of the saponified carotenoid mixture of the peel and pulp may be quantitatively rather similar. The percentages of the five fractions for the pulp were in fairly good agreement with those previously found for late-season orange juice (*1*), as included in Table II.

Most of the components obtained on chromatography of the five fractions obtained on countercurrent distribution were similar to those previously found in orange juice (*1–3*). Components not previously reported, including a number of stereoisomers, are listed in Table III together with spectral absorption maxima; the cis isomers may be artifacts. The tentative identifications were based on the countercurrent distribution fraction from which the compound was obtained, the behavior on chromatography, the spectral absorption maxima, the shape of the spectral absorption curve, and in some cases, hydrochloric acid–ether tests and behavior on iodine isomerization.

The approximate percentages of the components given in Table IV were based on the volumes of the solutions, the dilutions used in running the spectral absorption curves, and the absorbances of the highest peak of the curve. As the molecular extinction coefficients of many of the components are not known, no attempt was made to correct the above values accordingly. Components which were apparently cis isomers were combined with the corresponding all-trans isomers; the mutatoxanthins and lutexanthins include pairs of bands with practically identical spectral absorption curves.

In earlier work (*1*) on late-season Valencia orange juice, it was found that ζ- and β-carotenes occurred in similar amounts; in the present work the ζ-carotene was found in the pulp in about five times the amount of the β-carotene. Phytofluene also occurred in considerably larger relative amounts than found previously.

Table I. Fractions Obtained on Countercurrent Distribution of Orange Carotenoids with System Petroleum Ether–99% Methanol

(99 transfers)

Fraction	Unsaponified Pulp		Unsaponified Fresh Peel		Saponified Aged Peel	
	N_{100}[a]	%[b]	N_{100}[a]	%[b]	N_{100}[a]	%[b]
I No free hydroxyl	92	77	94	74	91	6
II A One free hydroxyl	66	16	54	24	58	13
II B One free hydroxyl and one ether group	..	..	..	..	39	5
III A Two or more free hydroxyls	13	7	4	2	8	50
III B Two or more free hydroxyls	..	..	..	..	2	26

[a] Position of maximum calculated on basis of 100 transfers.
[b] Calculated as β-carotene.

Table II. Fractions Obtained on Countercurrent Distribution of Saponified Orange Carotenoids

(System benzene–petroleum ether–87% methanol. 199 transfers)

Fraction	% as β-Carotene			Ratio % in Peel / % in Pulp
	Peel	Pulp	Juice (*1*)	
I + II Hydrocarbon + monol	12.8	26.7	19	0.48
III A Diol	4.9	12.3	22	0.40
III B Monoether diol	11.6	24.2	26	0.48
III C Diether diol	65.9	25.0	23	2.64
III D Polyol	4.8	11.8	10	0.41

Table III. Partial List of Carotenoids Obtained from Orange Peel and Pulp by Chromatography of Fractions Separated by Countercurrent Distribution

Fraction	Tentative Identification	Absorption Maxima[a]	Solvent
	Pulp		
II-1	*cis*-Hydroxy-α-carotene	469, 440, (421), 330	Pet. ether
II-4	Cryptoflavinlike	451, 425, 402	Pet. ether
III B-1	Antheraxanthin isomer	480, 453, 337	Benzene
III C-1	Violaxanthin isomer	469, 441, 417, 335, 320	Benzene
III C-3	*cis*-Lutcoxanthin a	455, 427, 403, 318, 303	Benzene
III C-4	*cis*-Lutcoxanthin b	455, 428, 405, 318	Benzene
III D-2	Sinensiaxanthin	419, 396, 373, 293	Ethyl alcohol
III D-3	*cis*-Sinensiaxanthin	414, 390, 370, 294	Ethyl alcohol
	Fresh Peel		
II-2	Cryptoxanthin epoxide	471, 444, 420	Pet. ether
II-4	Cryptoxanthin isomer	471, 444, (425), 334	Pet. ether
II-7	Cryptochromelike	422, 398, 376, 356	Pet. ether
III A-3	Capsanthinlike	(509), 479, 359	Benzene
	Aged Peel		
I-5	*cis*-ζ-Carotene	424, 398, 377, 298	Pet. ether
I-6	ζ-Carotene	426, 400, 378, 297	Pet. ether
II A-4	Cryptoflavinlike	453, 426, 403	Pet. ether
II B-1	Hydroxy-α-carotene furanoxidelike	446, 418, 398	Pet. ether

[a] Figures in parentheses indicate humps on spectral absorption curves.

The monol fractions, especially that from the peel, were somewhat more complex than previously reported for orange juice (*1*). Fractions which appeared to be mono-*cis*-hydroxy-α-carotene were obtained from both pulp (II-1) and peel. Bands with highest wave-length absorption maxima in petroleum ether of around 450 mμ were found in both peel and pulp carotenoids; the wave length of the maxima and the shape of the curves were similar to those of fractions previously identified as mutatoxanthins (zeaxanthin furanoxides), and likewise gave a light-blue color in the hydrochloric acid–ether test. Hence, these fractions were tentatively identified as cryptoxanthin furanoxide (cryptoflavin), which has been prepared synthetically by Karrer and Jucker (*4*) but apparently not found previously in nature. As pointed out in an earlier publication (*3*), values for spectral absorption maxima obtained with visual spectroscopes are often as much as 6 or 7 mμ higher than those obtained with photoelectric spectrophotometers. This accounts for the differences in values obtained in the present work from those given by Karrer and Jucker (*4*). The corresponding epoxide undoubtedly was present. Fraction II-2 (Table III) from the peel, which was eluted just ahead of cryptoxanthin, gave a light-blue color in the hydrochloric acid–ether test; on treatment of this fraction with hydrochloric acid in methanol and rechromatography, the main product had absorption maxima in petroleum ether at 450, 423, and 402 mμ, similar to the cryptoflavinlike fraction. This indicates that fraction II-2 was cryptoxanthin epoxide. The cryptoflavinlike fractions from both peel and pulp on chromatography were found on the column well above cryptoxanthin, as expected, as it was found (*3*) that an epoxide usually was eluted from the column slightly ahead of the parent desoxy carotenoid, whereas the isomeric furanoxide was eluted somewhat less readily. Fraction II-7 from the peel had absorption maxima a little below those reported for cryptochrome (cryptoxanthin difuranoxide) (*4*); this minor fraction was not pure. Fraction II-4 from the peel was tentatively identified as a stereoisomer (di-cis ?) of cryptoxanthin; it had a higher peak than the all-trans isomer at 334 mμ.

Fraction III A-3 was found only in the peel lipides; its occurrence on countercurrent distribution in fraction III indicated the probable presence of two hydroxyl groups with no other oxygen atom. The spectral absorption curve had a main maximum at 479 mμ in benzene, with an inflection at about 509 mμ. The shape of the curve and the wave length of the maximum were not changed by isomerization by light in the presence of iodine, an indication that this was not a poly-cis compound such as prolycopene (*8*). The spectral absorption curve was very much like that reported by Polgár and Zechmeister for capsanthin (*6*), which contains a ketone group and two hydroxyl groups; the behavior of capsanthin on countercurrent distribution is not yet known.

In the chromatography of the pulp carotenoids, the antheraxanthin (III B) and violaxanthin (III C) bands separated as usual into two bands—the upper of which in both cases apparently was the mono-cis isomer (*3*). The lower bands (III B-1 and III C-1 in Table III), contrary to previous experience, had lower wave-length absorption maxima than the upper, although the position of the cis peaks was unchanged. The results indicated that the lower band in each case, and especially with violaxanthin, must have contained di-cis or poly-cis isomers.

In earlier work (*3*) two luteoxanthins (flavoxanthinlike substances) with practically identical spectral absorption curves were found, with the highest wavelength maximum at 460 mμ in benzene, together with one fraction with wave lengths about 4 to 5 mμ lower. In the present work two consecutive bands (III C-3 and III C-4 from the pulp) were eluted which had highest wavelength maxima at 455 mμ. These observations are in agreement with the work of Strain (*7*), who reported two pairs of flavoxanthinlike pigments derived from violaxanthin and violeoxanthin (*cis*-violaxanthin ?), one pair of which had maxima 7 mμ shorter in wave length than the other.

In earlier work (*3*) sinensiaxanthin

Table IV. Carotenoids in Carotenoid Fractions of Orange Pulp and Fresh Orange Peel

(Assuming all constituents to have same specific extinction coefficient)

Constituent	Orange Pulp, %	Fresh Orange Peel, %
Phytoene	4.0[a]	3.1[a]
Phytofluene	13	6.1
α-Carotene	0.5	0.1
β-Carotene	1.1	0.3
ζ-Carotene	5.4	3.5
OH-α-Carotenelike	1.5	0.3
Cryptoxanthin epoxidelike	...	0.4
Cryptoxanthin	5.3	1.2
Cryptoflavinlike	0.5	1.2
Cryptochromelike	...	0.8
Lutein	2.9	1.2
Zeaxanthin	4.5	0.8
Capsanthinlike	...	0.3
Antheraxanthin	5.8	6.3
Mutatoxanthins	6.2	1.7
Violaxanthin	7.4	44
Luteoxanthins	17	16
Auroxanthin	12	2.3
Valenciaxanthin	2.8	2.2
Sinensiaxanthin	2.0	3.5
Trollixanthinlike	2.9	0.5
Valenciachrome	1.0	0.7
Sinensiachromelike	...	0.2
Trollichromelike	3.0	0.8

[a] Approximate values.

Figure 1. Countercurrent distribution of saponified orange peel and pulp carotenoids

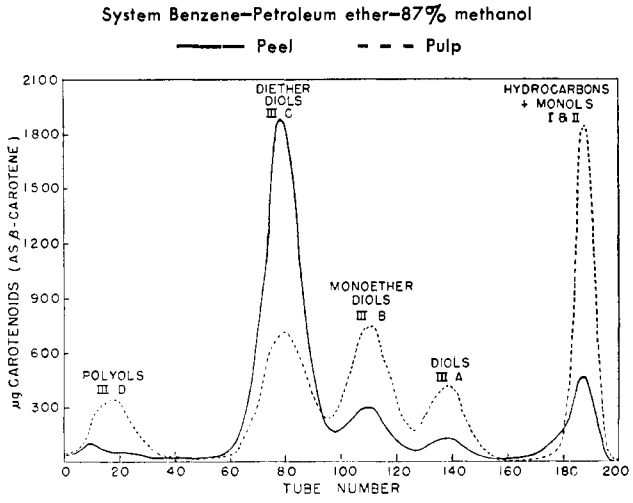
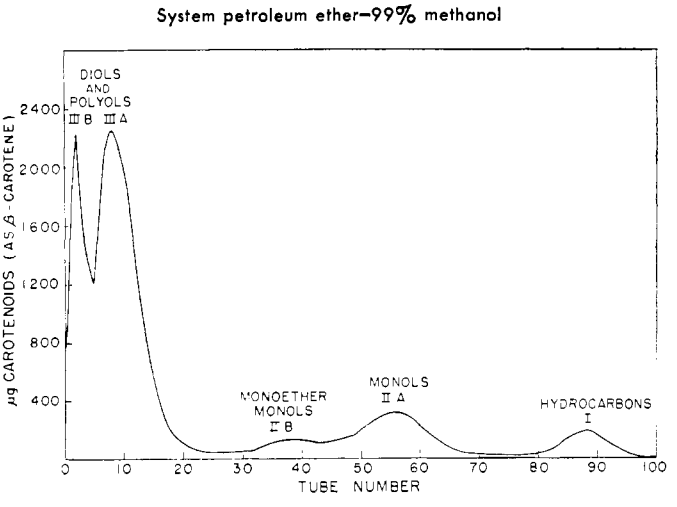


Figure 2. Countercurrent distribution of saponified carotenoids from aged orange peel



was obtained in only one form; in the present work this fraction (from either peel or pulp) was separated into two bands with similar spectral absorption curves, the first one eluted (III D-2, Table III) having absorption maxima several mμ higher than the second (III D-3). The latter probably was a mono-cis compound.

A comparison of the approximate percentages of the carotenoids of peel and pulp is given in Table IV, all probable stereoisomers being combined. The comparative values in most cases were in fair agreement. The peel carotenoids had a much higher concentration of violaxanthin than the pulp, but much less auroxanthin. More lutein than zeaxanthin was found in the peel. Considerably higher percentages of the trollixanthinlike and trollichromelike substances were found in the pulp. The method of preparation of the lipide extracts involved longer standing than in the usual procedure with juice (*1*), so that the epoxide-furanoxide isomerization apparently took place to a considerably greater extent, which accounts for the relatively higher values of mutatoxanthins, luteoxanthins, auroxanthin, and the trollichromelike fraction than in earlier work on juice (*3*). As a result of much lower acidity, these changes were much smaller in the peel lipides. It is probable that in both cases the furanoxides were found in larger proportions than were originally present in the fruit. However, the furanoxides on chromatography are usually much more readily separated from the corresponding desoxy carotenoid than are the isomeric epoxides; the finding of a furanoxide is thus a strong indication of the presence in the fruit originally of the isomeric epoxide.

Countercurrent Distribution and Chromatography of Saponified Carotenoids from Aged Peel. A fraction tentatively identified as cryptoxanthin furanoxide (cryptoflavin) was isolated from both orange peel and pulp carotenoids; cryptoflavin contains one hydroxyl group and one cyclic ether group and should be separable by countercurrent distribution from cryptoxanthin, which contains no ether group, when the system petroleum ether–99% methanol is used. Accordingly a 100-tube run was made with this system on a saponified lipide fraction from the same batch of peels which had been extracted after storage at 6° C. for 4 months, but still had a good color and aroma. Separation into five fractions occurred, as shown in Figure 2. The fraction labeled II B, with maximum at tube 38, occurred at about the place expected for cryptoxanthin furanoxide.

All five of the fractions were chromatographed. In fraction I the γ-carotene band was separated into two stereoisomers: I-5 and I-6 (Table III).

Fraction II A was somewhat simpler in composition than fraction II from fresh peel, but contained minor amounts of two components which were apparently cryptoxanthin furanoxide and a mono-cis isomer. Three components, all apparently furanoxides, were found in fraction II B. The two major components were very similar to the two fractions from II A, which were apparently cryptoxanthin furanoxide and a cis isomer thereof. The minor fraction (II B-1, Table III) had absorption maxima somewhat lower in wave length than the other two, and the shape of the curve was that of a carotenoid with the conjugated double band system entirely in the central chain, with much deeper minima between the maxima. This may have been a furanoxide of the hydroxy-α-carotene. The position of fraction II B on countercurrent distribution is supporting evidence for the identification of II A-4 (Table III) as cryptoxanthin furanoxide. In earlier work (*1*) in which no fraction resembling II A-4 was isolated, there was also no fraction corresponding to fraction II B found on countercurrent distribution.

Considerable difficulty was encountered in completely resolving fractions III A and III B by chromatography. Fraction III A apparently consisted of diols, while III B contained polyols, but the separation of III A and III B was only partial. Because of the complexity of the xanthophyll mixture of oranges, separation by countercurrent distribution before chromatography results in much better separation of the numerous constituents.

The spectral absorption curves of a number of the fractions obtained from both fresh and aged orange-peel carotenoids had maxima in benzene in the region 309 to 335 mμ. These maxima were high and rather narrow, often three and sometimes six times as high as the highest maximum in the visible region. In fresh peel they were found accompanying several diethertiol constituents, also a few polyols, especially violaxanthin and luteoxanthin fractions. In some cases the maximum fell at or very close to the major cis peak, but these maxima were not changed by light in the presence of iodine. These high maxima occurred at several wave lengths, mainly 312, 321, 327, and 335 mμ. In no case have these unusual maxima been found in fractions isolated from orange pulp or juice. The compounds responsible are not known, but may be sesquiterpenes or other components of the peel oil.

Part II – Carotenoids of Aged Canned Valencia Orange Juice

On storage of canned Valencia orange juice at 21° C. for one year, Mylne (*5*) found losses of up to 30% of the total carotenoid content, measured colorimetrically. The carotenoids of aged canned late-season Valencia orange juice, which had been stored at room temperature for 3 years, have now been examined. A very striking change was found to have taken place. None of the carotenoid epoxides (which in earlier work had been found to comprise about half of the total carotenoids) were detected, but the corresponding isomeric furanoxides were all found in much greater proportions than in fresh juice carotenoids. The diether diol fraction was relatively much smaller than in fresh juice, suggesting that violaxanthin, initially the principal constituent of this fraction, may have decomposed in other ways than by conversion to the isomeric difuranoxide auroxanthin. Only a minor part of the carotenoids were recovered as cis isomers, indicating that the loss in color of aged canned orange juice was not due to the formation of less highly colored cis isomers.

Experimental

Six lots of Valencia orange juice which had been canned (in citrus-enamel cans) in this laboratory in September and November of 1950, and stored at room temperature for 3 years were used. The juice still had a good color but had developed a very strong off-flavor. Three composite samples of the six lots were used in the isolation of the lipide fractions (*1*); two of these were then saponified.

Countercurrent distribution runs were made in the Craig apparatus as follows: (A) unsaponified and (B) saponified lipides, using the solvent system petroleum ether–99% methanol (1.8 to 1 by volume), with 99 transfers; and (C) saponified lipides, using the system benzene–petroleum ether–87% methanol (1:1:1.15 by volume), with 197 transfers. The color in each (99 transfer runs) or every other tube of the Craig apparatus was measured in an Evelyn photoelectric colorimeter, using a 440-mμ filter, and the results were calculated as β-carotene (*1*). The contents of the tubes were then combined into fractions, which were evaporated in vacuo and

the residues were dissolved in petroleum ether or benzene and chromatographed on columns of magnesia (Westvaco No. 2642) plus filter aid (1 to 1 by volume), using as eluents solutions of acetone or ethyl alcohol in petroleum ether. The fractions so obtained were evaporated in vacuo and dissolved in petroleum ether, benzene, or ethyl alcohol; spectral absorption curves were run in a Cary recording spectrophotometer. The relative amounts of the components so obtained were estimated from the absorbance of the solutions at the principal maxima, and from the dilutions that had been made.

Results and Discussion

Countercurrent Distribution of Unsaponified Lipides The total carotenoid content of the unsaponified lipide solution was equivalent to 23 mg. of β-carotene per liter of juice. The carotenoids were separated by countercurrent distribution with the system petroleum ether–99% methanol into three fractions as in previous work (*1*), which consisted of compounds containing 0, 1, and 2 or more free hydroxyl groups, respectively. The N_{100} values and the relative amounts of these three fractions (Table V) were similar to those previously found for unsaponified orange juice lipides (*2*), an indication that no significant amount of hydrolysis of the xanthophyll esters had occurred on the prolonged storage, and that there was no marked difference in the relative tability of the three major fractions as a whole under these conditions. The free xanthophyll fraction may have suffered somewhat greater losses than the esterified xanthophylls.

Fraction III, which was probably essentially free xanthophylls, was saponified and chromatographed, yielding eight components as listed in Table VI. A similar experiment had been carried out with the corresponding fraction obtained from frozen juice also from late-season fruit. The percentages of the various components found in this work (*2*) are listed in Table VI for comparison. The chief difference between the two is that for the frozen fresh juice, 26% of fraction III consisted of a trollixanthinlike epoxide, none of which was found for the aged canned juice. The latter contained 36% of trollichromelike substances (furanoxides), while only 12% of these were found in the fraction from fresh juice. This indicates that on prolonged storage, the trollixanthinlike epoxide was transformed completely to the corresponding furanoxide. The percentages of the other constituents of fraction III from the frozen fresh and aged canned juices agreed rather closely, indicating little change had occurred in them.

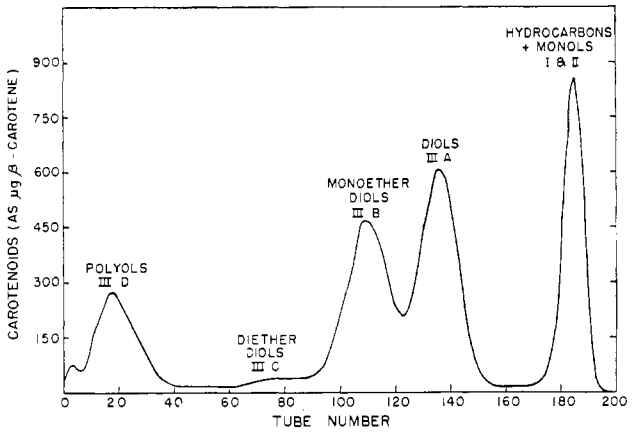


Figure 3. Countercurrent distribution of saponified carotenoids from aged canned Valencia orange juice

System benzene–petroleum ether–87% methanol. 197 transfers

Countercurrent Distribution of Saponified Lipides **Petroleum Ether–99% Methanol.** After saponification, the total carotenoid content was equivalent to 19 mg. (as β-carotene) per liter of juice, indicating a loss on saponification of about 17%, at least part of which was due to the formation of alkali-soluble acidic pigments (*2*). On countercurrent distribution a clean-cut separation into three major fractions occurred: (I) hydrocarbons, (II) monols, and (III) (diols and polyols) (Table V); the latter was partially separated into two parts (III and IV) with N_{100} values of 10 and 1, respectively.

Fractions I, II, and IV were investigated chromatographically. The separation of fraction IV was rather unsatisfactory, owing to the complexity of the mixture, but trollein, valenciachrome, auroxanthin, and the trollichromelike substance were apparently the major components, indicating fraction IV to consist mainly of polyols and diether diols.

The components obtained by chromatographing fractions I and II are listed in Table VII, and were essentially the same as found previously in fresh or frozen fresh juice. As in other late-season juices examined, the quantity of ζ-carotene was of the same order of magnitude as the β-carotene; the phytoene and phytofluene contents were about that of the α-carotene. Fractions 6 and 8 were judged to be cis isomers of the hydroxy-α-carotenelike substance and cryptoxanthin (fractions 7 and 9, respectively) by the shape of the curves and the position of the cis peaks. Treatment with light in the presence of iodine of fractions 6 and 8 resulted in an increase in wave length of the spectral absorption maxima in both cases to values practically identical with those obtained under similar conditions with fractions similar to 7 and 9, respectively, confirming the identifications. Fractions 10 and 11, which were obtained in

Table V. Fractions Obtained on Countercurrent Distribution of Aged Canned Orange Juice Carotenoids with System Petroleum Ether–99% Methanol

(99 transfers)

	Unsaponified		Saponified	
Fraction	N_{100}[a]	%	N_{100}	%
I No free OH	91	77.1	92	5.0
II One free OH	66	16.5	57	14.6
III > One free OH	17	6.4	10	63.6
IV > One free OH	..	...	1	16.9

[a] Position of maximum calculated on basis of 100 transfers.

Table VI. Carotenoid Components Obtained from Fraction III (Free Diols and Polyols) of Unsaponified Lipides from Aged Canned Valencia Orange Juice

Tentative Identification	Solvent	Absorption Maxima, Mμ	Approximate % Aged canned juice	Approximate % Frozen fresh juice (2)
Lutein	Benzene	486, 456, (432), 337	5	6
Zeaxanthin	Benzene	489, 461, (436), 344	13	9
Flavoxanthinlike	Benzene	461, 433, 407	2	1
Trollein	Ethyl alcohol	(474), 447, (426), 329	14	15
Mutatoxanthin (?)	Ethyl alcohol	449, 426, 316	9	7
cis-Trollichromelike	Ethyl alchool	444, 424, 314, 302	7 } 36	..
Trollichromelike	Ethyl alcohol	449, 423, 399, 313, 300	29 }	12
Auroxanthin	Ethyl alcohol	427, 403, 382, 297	19	22
Trollixanthinlike	...	...	0	26

small quantities, had spectral absorption curves similar to those of fractions previously found in early season peel and pulp, which had been tentatively identified as cryptoxanthin furanoxide (cryptoflavin) isomers. Similar fractions had not been obtained previously from late-season juices. It is probable that in the fresh or frozen juice, this fraction existed principally as the isomeric epoxide, which was probably overlooked because of the relatively small amount and the occurrence on the chromatogram between cryptoxanthin and the hydroxy-α-carotenelike substance. Fractions tentatively identified as cryptoflavin gave a pale-blue color in the hydrochloric acid–ether test, which could have been overlooked when solutions consisting principally of cryptoxanthin or hydroxy-α-carotene were tested.

Benzene–Petroleum Ether–87% Methanol. The distribution curve (Figure 3) with this solvent system was different from those previously obtained from fresh juice (*1*), in that the diether diol fraction (III C) was much smaller, while the other fractions were of the same order of magnitude as the corresponding ones from fresh juice. The N_{100} values and percentages of the several fractions, calculated as β-carotene, are given in Table VIII. The N_{100} values are close to those previously obtained with the corresponding fractions of fresh juice. Fractions III A, III B, III C, and III D were all chromatographed.

Fraction III A yielded lutein and zeaxanthin only, as in fresh juice (Table III). Fraction III B, unlike the corresponding fraction from fresh juice, contained no antheraxanthin, but consisted almost entirely of two bands similar to the *cis*-mutatoxanthin and mutatoxanthin b reported earlier (*3*). These substances are formed by the action of acid on antheraxanthin. The third, minor fraction (No. 14, Table VII), was not identified; the spectral absorption curve was not significantly changed by light in the presence of iodine. More recent work has indicated that this fraction was probably flavoxanthin (lutein furanoxide).

Fraction III C, which was much lower in amount than in earlier work with fresh juice, was found to consist principally of auroxanthin and a monocis isomer, no violaxanthin or luteoxanthins being found as in fresh juice. The percentage of auroxanthin was considerably lower than would have been expected if all of the violaxanthin and luteoxanthins had been converted to auroxanthin; hence it is probable that other changes occurred, possibly resulting in the formation of mutatoxanthins, trolleins, or trollichromelike substances.

Fraction III D was also different from the corresponding fractions found previously in fractions from fresh juice (*3*), no trollixanthinlike fraction, valenciaxanthin, or sinensiaxanthin being found (all three are epoxides). The corresponding furanoxides were found in all three cases: the trollichromelike fraction, valenciachrome, and sinensiachrome. Sinensiachrome had not previously been obtained from orange juice, but was formed as a product of the reaction of hydrochloric acid on sinensiaxanthin (*3*). The amount found in this instance was small. The substance trollein (*3*), apparently a desoxy derivative of the trollixanthinlike substance, as well as valenciachrome and the trollichromelike substance, were all found as two distinct, well separated bands with practically identical absorption curves; this phenomenon had previously been noted for other furanoxides—mutatoxanthins, luteoxanthins, and auroxanthin (*2*, *7*). Trollein, however gave no blue color in the hydrochloric acid–ether test; it is probably not a cyclic ether, but, judged by its behavior on chromatography and countercurrent distribution, very probably a polyol. The spectral absorption curve is very similar to those of lutein and antheraxanthin, suggesting that it may be derived from antheraxanthin by replacing the ether group with two hydroxyl groups.

Table VII. Carotenoid Components Isolated from Saponified Lipide Fraction of Aged Canned Valencia Orange Juice

Fraction	Tentative Identification	Solvent	Absorption Maxima, Mμ	Approx. %	
		Hydrocarbons (Fraction I)			
1	Phytoene	Pet. ether	(300), 288, 278	1.0	
2	Phytofluene	Pet. ether	367, 348, 333	0.8	
3	α-Carotene	Pet. ether	474, 444, 423, 333	0.7	
4	β-Carotene	Pet. ether	477, 451, (426), 338	2.1	
5	ζ-Carotene	Pet. ether	425, 400, 378, 298, 287	3.2	
		Monols (Fraction II)			
6	*cis*-OH-α-Carotene	Pet. ether	470, 442, 418, 332	0.6	2.9
7	OH-α-Carotene	Pet. ether	474, 446, 422, 333	2.3	
8	Poly-*cis*-cryptoxanthin	Pet. ether	470, 443, (420), 337	0.8	6.7
9	Cryptoxanthin	Pet. ether	478, 451, (426), 337	5.9	
10	*cis*-Cryptoflavin	Pet. ether	451, 425, 402	0.1	0.3
11	Cryptoflavin	Pet. ether	453, 426, 403	0.2	
		Diols (Fraction III A)			
12	Lutein	Benzene	486, 456, (431), 338	9.9	
13	Zeaxanthin	Benzene	491, 463, (435), 346	14.2	
		Monoether Diols (Fraction III B)			
14	Flavoxanthinlike	Benzene	458, 432, 410, 312	0.6	
15	*cis*-Mutatoxanthin	Benzene	463, 437, (413), 323	16.8	30.9
16	Mutatoxanthin b	Benzene	466, 438, (414), 324	14.1	
		Diether Diols (Fraction III C)			
17	*cis*-Auroxanthin	Benzene	433, 407, 385, 303, 292	0.6	7.2
18	Auroxanthin	Benzene	437, 411, 388, 302, 292	6.6	
		Polyols (Fraction III D)			
19	Unknown	Ethyl alcohol	394, 374, (358)	1.1	
20	Trollein a	Ethyl alcohol	474, 445, 423	1.9	
21	Trollein b	Ethyl alcohol	474, 446, 424, 333	2.1	4.7
22	*cis*-Trollein (?)	Ethyl alcohol	470, 443, 420	0.7	
23	Valenciachrome a	Ethyl alcohol	368, 348, 333	1.0	2.5
24	Valenciachrome b	Ethyl alcohol	368, 348, 333	1.5	
25	Trollichromelike a	Ethyl alcohol	449, 423, 399, 314, 302	5.2	7.0
26	Trollichromelike b	Ethyl alcohol	448, 423, 397	1.8	
27	Sinensiachrome	Ethyl alcohol	396, 374, 353	0.2	

Table VIII. Fractions Obtained on Countercurrent Distribution of Saponified Aged Canned Orange Juice Carotenoids with System Benzene–Petroleum Ether–87% Methanol

(197 transfers)

Fraction	N_{100}[a]	% as β-Carotene	
		Present	Previous results
I + II Hydrocarbons + monols	95	23.5	19–27
III A Diols	70	30.8	12–22
III B Monoether diols	56	27.3	24–26
III C Diether diols	41	2.2	23–25
III D Polyols	9	16.1	10–12

[a] Position of maximum calculated on basis of 100 transfers.

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MOISTURE DETERMINATION

Determination of Water by Nuclear Magnetic Absorption in Potato and Apple Tissue

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The measurement of nuclear magnetic resonance absorption of hydrogen nuclei in water provides a rapid method for the determination of the water content of potato tissue without reference to other standard moisture procedures. In the case of apple tissue, the same procedure can be used, provided correction is made for soluble solids. Using the method previously applied to starch suspensions, the results obtained on water content of potatoes by the nuclear magnetic resonance absorption method agree with results obtained by the vacuum oven method; for apples, the results are too high because of the contribution to the nuclear magnetic absorption of hydrogen nuclei in the soluble solids. In these cases moisture can be determined if the contributing soluble constituents are known, or by means of a calibration curve.

A METHOD for the quantitative measurement of hydrogen in liquids and suspensions by nuclear magnetic resonance absorption was presented in a previous paper (*3*). When applied to aqueous suspensions of starch, it was shown that the method measured only the hydrogen in the aqueous phase and could be used to determine the amount of water in starch suspensions to within about 2%. The results obtained for starch suspensions indicated that the same method might be useful for the measurement of the water content of fruit and vegetable tissues or similar biological materials on an absolute basis. In the present paper these possibilities are investigated for two types of tissue, potato and apple. The essential features of the method are given in the previous paper (*3*).

Experimental

The methods of measurement and apparatus are the same as those used in the previous study (*3*). Potato and apple were chosen because they are typical of many others for which a rapid method of moisture measurement would be of value; moreover, they are sufficiently large to provide test specimens in the form of single cylindrical pieces about 2 cm. in diameter, 3.24 cm. in length for potato, and 1.93 cm. in length for apple.

The cylindrical specimens of tissue were obtained by means of a cork-boring machine. The cylinders of tissue were inserted into a jig consisting of a close-fitting glass cylinder. The ends of the tissue were cut off flush with the ends of the glass cylinder.

The maximum variation in the volume of the specimens was estimated to be about 2%. This variation was due primarily to variations in diameter. The volume of the cylindrical glass jig is assumed to be the maximum volume of the test specimen.

If the magnetic fields employed in the magnetic absorption measurement were uniform, no error would result from the fact that the actual volume of the test specimen is smaller than the assumed volume—i.e., the volume of the glass jig. For example, if the volume of the jig is assumed to be V_a, and the specimen volume is $V_0 < V_a$, then the observed signal is

$$(D_{\max_0})_0 = (D_{\max_0})_a \times (V_0/V_a) \quad (1)$$

and the apparent bulk density is

$$\rho_0 = \rho_a(V_0/V_a) \quad (2)$$

Now the water content (w.c.) (per cent by weight), is expressed as

$$\text{water content} = [(D_{\max_0})_0/(D_{\max_0})_{\text{water}}] \times (\rho_{\text{water}}/\rho_0) \quad (3)$$

From Equations 1, 2, and 3

$$\text{water content} = [(D_{\max_0})_a/(D_{\max_0})_{\text{water}}] \times (\rho_{\text{water}}/\rho_a) \quad (4)$$

Thus if Equation 1 is true—i.e., signal strictly proportional to volume—the error in water content due to an error in the specimen volume would be zero. However, as the magnetic field employed in the nuclear magnetic resonance measurements was not uniform, Equation 1 is only approximately true. A reasonable estimate is that the maximum error in water content is probably not greater than 1% for a 2% volume error.

The nuclear magnetic resonance absorption experiments were made at room temperature (26° ± 2° C.). The water content of the test specimens was also determined by standard vacuum oven procedures.

As in the previous paper, the peak absorption signal, $D_{\max}$, was determined for several values of the radio-frequency magnetic field intensity, H_1, in the range 2 to 6 × 10^{-4} gauss. Graphs of $D_{\max}$ *vs.* H^2 were extrapolated linearly to obtain $(D_{\max})_0$. In order to standardize the apparatus and check over-all performance, a similar determination was made for distilled water at various times