and winter months can be seen in Table III. According to the *t*-test, the differences from one season of harvest to the other were significant; fruits from summer contained 0.8 to 3.0% more sugar and yielded correspondingly higher RDS's, from 1.4 to 3.8% more, than winter fruits. A variation among orchards in the degree of difference between summer and winter fruits is apparent, but the trend in each was the same. The coefficient of correlation was in this evaluation only 0.610; nevertheless it was significant at the 95% level of probability. The regression line, with its relatively broad confidence bands, is seen in Figure 2. The difference between the RDS and the sugar content was about 1%.

Sugar is indeed the predominant water-soluble substance in papaya fruit; it comprises about 90% of the sap solutes. Thus, the correlation between juice soluble solids and sugar content is expectedly excellent, and the former is applicable as an indicator of the latter in certain situations. In variety trials, those yielding fruits with low juice RDS's—e.g., the first three in Table I—may be quickly eliminated from further screening. According to the Table II data, the sugar level in green fruits is markedly lower than in those which have attained a degree of yellowness. Growers ordinarily harvest fruits, particularly those which are intended for the more distant markets, when still green. A minimum content of sugar, or a standard of quality, in the marketed fruit may be assured by including, perhaps in the packing step, a systematic examination of RDS's. The critical solid content will have to be agreed upon first.

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BELL PEPPER CAROTENOIDS

The Carotenoids of Green Bell Peppers

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The most abundant carotenoid in green bell peppers was lutein, with beta-carotene, violaxanthin, and neoxanthin also as major pigments; minor pigments included phytoene, phytofluene, alpha-carotene, and zeta-carotene. No keto carotenoids such as capsanthin or capsorubin were found, nor was capsolutein, a lutein-like pigment occurring in place of lutein in the ripe red fruit.

THE CAROTENOID MIXTURE in red bell peppers (*Capsicum annuum*) (*5*) was quite complex and contained a number of pigments not found in any of the numerous other fruits examined in this laboratory. Seven of these carotenoids contained keto groups, including capsanthin and capsorubin, the structures of which were shown recently (*7, 9*) to contain one and two cyclopentane rings, respectively. At least six other pigments apparently also contained cyclopentane rings, including capsolutein, a lutein-like pigment for which the structure 6'-deoxocapsanthin was proposed (*5*). No lutein was found. Capsolutein was indistinguishable from lutein in visible and ultraviolet absorption spectra, and differed only slightly in behavior on countercurrent distribution, but unlike lutein did not contain an allylic hydroxyl group.

The carotenoids of green bell peppers have now been investigated, to see if any of the keto carotenoids found in red bell peppers were present, and in particular to ascertain if lutein and/or capsolutein occurred. Another object was to obtain information on the carotenoids in green fruit, of which green bell peppers are a readily available example.

Experimental

Three lots of green bell peppers were obtained at a local market in April, June, and July. One kilogram (of each lot) of destemmed fruit was blended with 1 liter each of water and methanol, and 10 grams of magnesium carbonate; 100 grams of Celite 503 was then added and the mixture filtered on a Büchner funnel precoated with filter aid. The filter cake was worked up as previously described (*8*), including saponification.

Four solvent systems were used in countercurrent distribution runs in a Craig apparatus; systems I, II, and III were previously described (*3, 4*). System IVB (hexane and 70% methanol, 1 to 1 by volume) is similar to system IV (*4*). Aliquots of certain fractions obtained on countercurrent distribution were chromatographed on magnesia (Sea Sorb 43) 14 by about 90 mm., without a diluent. The magnesia was added to a column partially filled with hexane; air pressure was applied to the top of the column to ensure even packing, and also that the top be level. A topping of about 10 mm. of anhydrous sodium sulfate was then added in a similar manner (*6*). Spectral data were obtained with a Beckman DK-2 recording spectrophotometer. The test for allylic hydroxyl groups was previously described (*5*).

Results and Discussion

The total carotenoids obtained from the three lots of green peppers were 10.6 (April), 11.2 (June), and 9.0 (July) mg. per kg. (measured in an Evelyn photoelectric colorimeter at 440 mμ and calculated as beta-carotene); these values are much lower than those obtained with red bell peppers (248 and 127 mg. per kg.) (*5*).

Table I. Fractionation of Carotenoid Mixtures from Bell Peppers and Leaves by Means of Countercurrent Distribution with Solvent System I[a]

Source	Fractional Composition, %		
	I[b]	II	III
Green bell peppers	13.5	1.3	85.1
Red bell peppers	11.3	4.3[c]	84.3
Valencia orange leaves	25.6	1.7	72.7
Pyracantha leaves	35.9	2.0	62.1

[a] Hexane—99% methanol.
[b] I-hydrocarbons, II-monols, III-diols and polyols.
[c] Contained 3.3% of monols (II A) with N_{100} of 56, and 1.0% of monoketo monols (II B) with N_{100} of 38.

Table II. Fractionation by Countercurrent Distribution of Diol-Polyol Carotenoid Fractions

Source	Solvent System	Fractional Composition, %			
		IIIA[a]	IIIB	IIIC	IV
Green bell peppers	IVB[b]	50.4	0.6	31.1	17.9
Valencia orange leaves	II[c]	62.6	...	22.4	15.0

[a] IIIA-diols, IIIB-monoepoxide diols, IIIC-diepoxide diols, IV-polyols. [b] Hexane–70% methanol. [c] Hexane–benzene–87% methanol, 1 to 1 to 1.15 by volume.

Table III. Carotenoids Obtained from Green Bell Peppers

	Carotenoid	Spectral Absorption Maxima, mμ[a]	Approximate Percentage of Total Carotenoids
I-1	Phytoene	(299)[b], 285	1.4
I-2	epsilon-Carotene[c]	467, 437, (410)	0.03
I-3	Phytofluene	365, 349, 331	0.24
I-4	alpha-Carotene	472, 443, 418, 330	0.4
I-5	beta-Carotene	477, 448, (423), 338	13.4
I-6	zeta-Carotene	423, 397, 376	0.4
II-1	Unknown	447, 420, 395	0.22
II-2	Hydroxy-alpha-carotene[d]	472, 443, 418, 334	1.2
II-3	Cryptoxanthin[e,f]	472, 445, (422), (340)	0.5
IIIA-1	Unknown	481, 446	0.15
IIIA-2	Unknown	426, 402, 377	0.18
IIIA-3	Lutein[g]	487, 455, 431, 338	40.8
IIIA-4	Zeaxanthin[f,h]	487, 458, (434), 346	0.6
IIIC-1	Unknown	427, 404, 381	0.23
IIIC-2	Violaxanthin	483, 450, 424, 336, 321	13.8
IIIC-3	Luteoxanthin[a]	458, 429, 405, 318, 304	4.0 } 6.9
IIIC-4	Luteoxanthin[h]	457, 429, 405, 318, 304	2.9
IIIC-5	Unknown	485, 455, 432, 342	0.6
IIIC-6	Auroxanthins	434, 407, 386	1.0
IV-1	Unknown	431, 405, 384	0.9
IV-2	Neoxanthin[i]	477, 446, 420, 335, 320	15.1
IV-3	Neochrome[a]	457, 429, 405, 317, 304	0.7 } 1.8
IV-4	Neochrome[h]	456, 428, 404, 317, 304	1.1

[a] Fractions I and II in hexane, others in benzene. [b] Values in parentheses represent inflections or shoulders on spectral absorption curves. [c] Tentative identification. [d] N_{100} value of 56 of fraction II in solvent system I indicates fraction consists of 3-hydroxy-carotenes. [e] Band redder than II-2, spectral absorption curve had much less fine structure. [f] Apparently consisted, at least in part, of cis-isomers. [g] N_{100} value of 71 in solvent system II of fraction IIIA in good agreement with value obtained with crystalline lutein. [h] Band redder than IIIA-3, spectral absorption curve had much less fine structure. [i] Identification confirmed by N_{100} value of 64 in solvent system III.

Countercurrent Distribution.

Solvent system I was used to separate the carotenoid mixture from green bell peppers into three fractions—hydrocarbons, monols, and diols and polyols (Table I); comparable data are also given for red bell peppers and two species of leaves. The fractional compositions of the green and red bell pepper carotenoids were similar except in the latter the percentage of monols (including monoketo monols) was several times greater. The results with the green bell peppers were similar to those obtained with leaves except that the leaves contained much larger proportions of hydrocarbons (mainly beta-carotene).

Solvent system IVB was used to further fractionate the diol-polyol fraction of green bell peppers (Table II). Comparative data are given for Valencia orange leaves but not for red bell peppers; in the latter, the distribution was complicated by the occurrence of the monoketo diol capsanthin and the diketo diol capsorubin as major constituents. The distribution pattern of the diol-polyol fraction from green bell peppers was similar to that of the corresponding fraction from green leaves.

To obtain larger quantities of material for chromatography, a 100-transfer run using solvent system II was made on another portion of total carotenoid extract from green bell peppers, and the following fractions were examined chromatographically: hydrocarbon-monol (I-II), diol (III A), diepoxide diol (III C), and polyol (IV). The monoepoxide diol (IIIB) was omitted because of the very small amount. Substances obtained are listed in Table III.

Hydrocarbons (I). In the chromatography of fraction I–II, benzene in hexane (14, 20, 30, and 45%) was used to elute the hydrocarbons (I), then ethanol in hexane (0.5, 0.7, 1.0, and 1.4%) to elute the monols (II). Beta-carotene was the principal constituent of the hydrocarbon fraction; there were much smaller amounts of phytoene,

phytofluene, zeta- and alpha-carotenes. The phytofluene band also had a very small amount of absorbance in the visible region, with maxima at 468 and 436 mμ, possibly due to epsilon-carotene; this differs from beta-carotene in having alpha-ionone rings instead of beta-ionone rings at both ends of the molecule. A pigment resembling epsilon-carotene was found in tomato fruits by Trombly and Porter (11); it occurred on the column between phytoene and phytofluene.

Monols (II). On elution with ethanol in hexane, the first fraction (II-1) contained a relatively large amount of solids, probably sterols; this fraction may have contained a cis-mutatochrome (beta-carotene-5,8-epoxide) or alpha-carotene-5,8-epoxide. Two distinct minor bands followed, an orange one below and a redder one above. The orange band was identified as hydroxy-alpha-carotene, the upper as cryptoxanthin; the latter apparently consisted, at least in part, of cis-isomers which may

have been artifacts. The spectral absorption curve of the upper had much less fine structure, and had a cis-peak at a somewhat higher wave length.

Diols (IIIA). The major pigment of this fraction, and the most abundant by far, was identified as lutein and not capsolutein. On treating with hydrochloric acid in methanol and subjecting the product to a countercurrent distribution run with system I, nearly all of the material occurred as a fraction with N_{100} value of 40, in good agreement with the prior result of 38 for lutein monomethyl ether. The N_{100} values of lutein and capsolutein in this system were both 10, but the N_{100} value of capsolutein was unchanged after treatment with hydrochloric acid in methanol (5).

A very minor band on the column (IIIA-1) below lutein had spectral absorption maxima at 481 and 446 mμ. This may possibly have been the same as a nonepoxide diol found in red bell peppers (5), and tentatively identified as 6,6'-dideoxocapsorubin.

Above the lutein band was a much smaller, redder band (III A-4) which had similar spectral absorption maxima except the cis-peak was at 346 mμ instead of 338; the spectral absorption curve had much less fine structure. This was identified as zeaxanthin, and apparently consisted, at least in part, of cis-isomers which may have been artifacts.

Diepoxide Diols (IIIC). The major band of this fraction was violaxanthin (zeaxanthin-5,6,5',6'-diepoxide); it was accompanied by smaller amounts of the corresponding 5,6,5',8'-diepoxide isomers, the luteoxanthins, and by a much smaller amount of the 5,8,5',8'-diepoxide isomers, the auroxanthins.

Below the yellow auroxanthin band was a minor red band (IIIC-5) which had spectral absorption maxima near those of lutein. It was eluted much less readily than zeaxanthin and may have been a polyol such as hydroxycapsolutein (5).

Polyols (IV). The first band eluted from the column had pronounced spectral absorption maxima at 431, 405, and 384 mμ; these were only slightly changed on hydrochloric acid treatment, indicating that this substance was not a 5,6-epoxide such as sinensiaxanthin found in oranges (7). This band was eluted from a column much more rapidly than the auroxanthin band in fraction IIIC. The main constituent was neoxanthin, a 5,6-epoxide polyol of incompletely known structure, with much smaller amounts of the corresponding 5,8-epoxide isomers, the neochromes. When the major band (IV-2) was subjected to a countercurrent distribution run with system III (hexane-acetone-13% methanol), the N_{100} value was 64, in good agreement with previous results of 61-62 for neoxanthin from leaves.

As shown in Table III, lutein was the most abundant carotenoid, with somewhat smaller amounts of beta-carotene, violaxanthin, and neoxanthin. These are also the principal constituents of leaf carotenoids (10). Cholnoky et al. (2) recently investigated the carotenoids of green paprikas. Lutein was the most abundant constituent, with lesser amounts of beta-carotene, violaxanthin, "foliaxanthin," and "foliachrome." Small amounts or traces of beta-carotene-5,6-epoxide, mutatoxanthin, antheraxanthin, and apparently cryptoxanthin were also found. Three of the last four were not found in green peppers in the present work, two of which would

be in the monoepoxide diol fraction (IIIB) which was not chromatographically investigated. Cholnoky et al. did not report a number of the minor constituents found in the present work, including phytoene, phytofluene, alphacarotene, zeta-carotene, hydroxy-alphacarotene, and zeaxanthin. "Foliaxanthin," a 5,6-epoxide, is apparently the same as neoxanthin; "foliachrome," reported by Cholnoky et al. as being the corresponding 5,8-epoxide, may be the same as neochromes, or, since it was found on the column below "foliaxanthin," possibly the same as luteoxanthins.

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HEAT EFFECTS ON SOYMILK

Indices of Protein Quality in Dried Soymilks

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THE MAINTENANCE and evaluation of the nutritional quality of soybean products are of importance since such products often make up a significant part of an individual's food intake. This is the case when they are used as substitutes for animal milks in infant feeding and when they are used to elevate the protein content of otherwise inadequate diets. The use of animal feeding tests to obtain

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indices of protein quality (13) provides the most direct and reliable experimental evaluations of the products, but such tests are expensive and time consuming. Simpler and quicker methods for the control of the manufacture and procurement of soy products are widely recognized as being of value in ensuring high quality in the finished products (14).

For soybeans to provide a maximal contribution to nutrition, deleterious substances must be removed from the raw

beans. Fortunately, through the application of heat, it is possible to inactivate such harmful materials as the trypsin inhibitors (11). To evaluate the adequacy of heat treatments a number of tests have been devised. Examples are the measurement of the extent of inactivation of urease (3) or trypsin inhibitors (11), the lowering of soluble nitrogen (16) or alkali-soluble solids (14).

Heating of soybeans introduces the possibility that some of the nutrients may