mainly to the formation of colorless low molecular weight derivatives, while antioxidants slow the process by blocking some of the steps in the oxidative process.

During storage (Fig. 7), BHT was found to be the best long-term protective agent for β-carotene; 500 ppm α-tocopherol or deodorized rosemary oleoresin gave similar moderate effects. A dose of 250 ppm of these two is not sufficient in the drastic conditions of extrusion cooking.

Conclusion

Studies of the behavior of purified all-*trans*-β-carotene in extrusion cooking have shown that this type of treatment leads to extensive degradation of the molecule. The fraction of residual nonvolatile compounds at extruder output accounts for only 10 to 20% of the initial pigment content. It is to be noted that in commercial preparations of β-carotene for industrial use in which the colorant is coated in a matrix, losses are much lower.

Several types of chemical reactions are revealed, including multiple isomerizations on certain trans double bonds leading to modifications of the spatial conformation of the molecule and undoubtedly modifying its stability. In addition, oxidations of the β-carotene molecule occur (epoxy formation, hydroxylation, ketone function). Finally, oxidative rupture progressively shortens the unsaturated chain.

The rates of these degradation phenomena can be decreased by the presence of antioxidants, provided the compounds themselves are heat stable.

[14] Natural Sources of Carotenoids from Plants and Oils

By AUGUSTINE S. H. ONG and E. S. TEE

Introduction

A large number of pigments in living organisms—carotenoids, chlorophylls, anthocyanins, and porphyrins—provide the rich variety of color in nature. The carotenoids, believed to have derived their name from the fact that they constitute the major pigment in the carrot root, *Daucus carota*, are undoubtedly among the most widespread and important. This group of pigments is found throughout the plant kingdom (although their presence is often masked by chlorophyll) and in insects, birds, and other animals. These pigments provide a whole range of light yellow to dark red colorings; and, when complexed with proteins, they produce green and blue color-

ations. Thus, a wide variety of foods and feeds, e.g., yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobster, salmon, trout, and yellow corn, owe their color principally to carotenoids, as do certain food color extracts from natural sources such as palm oil, paprika, annatto, and saffron.[1-3]

Aside from providing aesthetic qualities as colorants in the plant and the animal kingdoms, carotenoids, synthesized exclusively by photosynthetic microorganisms and by members of the plant kingdom, play a fundamental role in metabolism. Most importantly, the carotenoids serve the animal kingdom as sources of vitamin A activity.

The main source of vitamin A in the diet of the rural communities in developing countries is carotenoids, the precursors of vitamin A, from vegetables and fruits. Preformed vitamin A found in meat, liver, and eggs is frequently out of reach of the economically deprived. Many of these countries have abundant vegetation, which is a rich source of carotenoids, so there should indeed be no vitamin A deficiency problem. However, vegetable consumption among young children is known to be low and whatever small amounts of carotenoids are ingested, together with a diet poor in fat and protein, makes absorption of these precursors of vitamin A poor. At the same time, it is also not clear which fruits and vegetables are richest in β-carotene. These are obviously important areas for studies and intervention.[4]

A new frontier in carotenoid research has been the examination of a possible association between carotenoids and the development and prevention of cancer. In addition to their role as vitamin A precursors, carotenoids are known to be able to act as antioxidants, and to inactivate (quench) highly reactive chemical species such as singlet oxygen, triplet photochemical sensitizers, and free radicals, which would otherwise induce potentially harmful processes (e.g., lipid peroxidation). Through these actions, it has been suggested that carotenoids may play important roles in cancer causation and prevention.[5-9]

[1] B. Borenstein and R. H. Bunnell, *Adv. Food Res.* **15**, 195 (1966).
[2] B. C. L. Weedon, *in* "Carotenoids" (O. Isler, H. Gutmann, and U. Solms, eds.), pp. 29–59. Birkhäuser, Basel, 1971.
[3] J. C. Bauernfeind, *J. Agric. Food Chem.* **20**, 456 (1972).
[4] E. S. Tee, *CRC Crit. Rev. Food Sci. Nutr.* **31**, 103 (1992).
[5] R. Peto, R. Doll, J. D. Buckley, and M. B. Sporn, *Nature (London)* **290**, 201 (1981).
[6] J. A. Olson, *J. Nutr.* **116**, 1127 (1986).
[7] N. J. Temple and T. K. Basu, *Nutr. Res.* **8**, 685 (1988).
[8] N. I. Krinsky, *Clin. Nutr.* **7**, 107 (1988).
[9] G. W. Burton, *J. Nutr.* **119**, 109 (1989).

Several lines of evidence have suggested that important relations exist involving carotenoids and cancer.[8,10-12] These include evidence of the effects of carotenoids in experimental cancer, epidemiological evidence suggesting associations between reduced risk of cancer and carotene status, and findings from cancer treatment and prevention trials. The possibility that carotenoids may play a role in the etiology and prevention of cancer has added a new, exciting dimension to the study of these nutrients in human nutrition.

Analysis of Carotenoids in Plant Sources

Particular emphasis has been placed on understanding the types and concentrations of carotenoids in foods. It is now apparent that previously reported values of vitamin A activity in food composition tables may be unreliable because the methodologies used were not sufficiently discriminative and thus had included carotenoids that do not possess vitamin A activity. Advances in studies into the structures and properties of various carotenoids have shown that only a handful of the hundreds of carotenoids occurring in nature possess vitamin A activity. Some of these may occur in higher concentrations than β-carotene, the most potent precursor of vitamin A. The identification and the quantitation of such carotenoids devoid of vitamin A activity are also important, as it has been suggested that, like β-carotene, they may also be associated with lower cancer risk. It is now evident that epidemiological studies associating cancers with diet would require more accurate data on carotenoid content of foods. Currently, association is on a broad basis, linking vegetable consumption or "carotene" content of foods to risk of developing cancer. Improved analytical methods would greatly help to identify the compounds of interest, not only in foods but in blood serum as well.

In cognizance of the need for more accurate analysis of carotenoids, the International Vitamin A Consultative Group (IVACG) has called on national laboratories to develop techniques for determining accurately the provitamin A content of native fruits and vegetables in order to support vitamin A deficiency prevention and control programs.[13] The group also has emphasized that because carotenoids may be protective against some

[10] M. M. Mathews-Roth, *Pure Appl. Chem.* **57**, 717 (1985).
[11] R. G. Ziegler, *J. Nutr.* **119**, 116 (1989).
[12] C. M. Williams and J. W. Dickerson, *Nutr. Res. Rev.* **3**, 75 (1990).
[13] K. L. Simpson, S. T. C. Tsou, and C. O. Chichester, "Biochemical Methodology for the Assessment of Carotenes," International Vitamin A Consultative Group, Washington, D.C., 1987.

forms of cancer, the quantitation of these compounds in foods assumes even broader importance. The lack of information on the amount and form of carotenoids in existing food composition databases has also been highlighted.[14] Although high-pressure liquid chromatography (HPLC) has been proposed as an efficient tool for the separation and quantitation of carotenoids, the IVACG recognizes that there are as yet limited data on the carotenoid content of foods using this technique.

The analysis of carotenoids and retinoids is complicated and beset with various problems because of the large number of naturally occurring carotenoids (as many as 600 have been reported). The occurrence of cis and trans isomers of carotenoids further complicates the analysis. The composition and content of carotenoids in various plant materials vary widely. Not all of the naturally occurring carotenoids are precursors of vitamin A, and for those with provitamin A activity, the biological activity varies widely.

It is, therefore, rather difficult to obtain accurate data on carotenoid content and composition. The literature abounds with attempts at improving the analytical techniques. The procedure should be able to remove interfering compounds effectively, to separate the carotenoids, and to quantitate them accurately. There has been much work on method development and improvement in this field.[15] A wide variety of separation, detection, and quantitation procedures have been used in studies of carotenoids and retinoids. An early technique used for the separation of carotenoids in plant materials, mainly in the 1960s, was countercurrent distribution. A few early studies using paper chromatography were also reported. In the 1970s, studies of carotenoids and retinoids using gas–liquid chromatography and gel-permeation chromatography were introduced and a number of studies employed thin-layer chromatography (TLC) as a single technique for the separation of carotenoids in plant materials. Thin-layer chromatography was also used by several investigators in combination with other separation techniques. The procedure was less widely used in studies of retinoids. Adsorption open-column chromatography, utilizing primarily descending, gravity-flow columns, was widely used for the study of carotenoids and retinoids in foods, even in the 1960s. This procedure remained very much in use in the 1970s and 1980s. Since the late 1970s, however, HPLC has become a widely used procedure for the separation of

[14] B. A. Underwood, M. Chavez, J. Hankin, J. A. Kusin, A. Omololu, F. Ronchi-Proja, R. Butrum and S. Ohata, "Guidelines for the Development of a Simplified Dietary Assessment to Identify Groups at Risk for Inadequate Intake of Vitamin A," International Vitamin A Consultative Group, Washington, D.C., 1989.

[15] E. S. Tee and C. L. Lim, *Food Chem.* **41**, 147 (1991).

carotenoids, mainly because the technique effects rapid separation, it is nondestructive and, more importantly, better resolution is achieved. The ability of HPLC to separate rapidly and to quantitate various carotenoids, at least in standard preparations, has been demonstrated. Its application to the analysis of foods, however, is still being developed and improved.

Many investigators have shown that the use of other techniques in combination with HPLC will greatly enhance the usefulness of the procedure. The combined use of open-column chromatography, TLC, and ultraviolet–visible (UV–VIS) absorption spectra, for example assists in the identification and confirmation of carotenoids.

Natural Sources of Carotenoids from Plants and Oils

The ability of organisms to produce carotenoids seems to have developed at an early stage in evolution. Photosynthetic bacteria, the algae, spore-bearing vascular plants, and the higher plants preserve this capability. Animals, certainly those of the higher orders, are not capable *do novo* carotenogenesis and are dependent for their carotenoids on those preexisting in their diet.[2] This chapter deals mainly with those carotenoids present in higher plants, which are the more important food sources.

Between 1933 and 1948, the number of known naturally occurring carotenoids increased from 15 to about 80, and rose sharply to about 300 some 20 years later.[16] In 1986 Goodwin[17] reported that over 500 of them were known. Most of these, however, are xanthophylls, which are oxygenated carotenoids. The common name and the semisystematic equivalent, together with the structure of about 300 natural carotenoids, have been listed in the 800-reference publication of Straub,[18] who updated the list to 400 compounds in a 1600-reference monograph.[19] Bauernfeind[3] tabulated the occurrence of some 40 common carotenoids in various foodstuffs.

It has been estimated that nature produces about 100 million tons of carotenoid pigments per year.[20] Most of this output is in the form of four major carotenoids, namely, fucoxanthin, the characteristic pigment of many marine algae and the most abundant natural carotenoid, and lutein, violaxanthin, and neoxanthin,[2] the three main carotenoids in green leaves. All other carotenoids are produced in relatively small amounts. However,

[16] O. Isler, "Carotenoids" (O. Isler, H. Gutmann, and U. Solms, eds.), pp. 11–27. Birkhäuser, Basel, 1971.
[17] T. W. Goodwin, *Annu. Rev. Nutr.* **6**, 273 (1986).
[18] O. Straub, *in* "Carotenoids" (O. Isler, H. Gutmann, and U. Solms, eds.), pp. 771–850. Birkhäuser, Basel, 1971.
[19] O. Straub, "Key to Carotenoids: Lists of Natural Carotenoids," Birkhäuser, Basel, 1976.
[20] O. Isler, R. Ruegg, and U. Schwieter, *Pure Appl. Chem.* **14**, 245 (1965).

some, like β-carotene and zeaxanthin, occur very widely and others, such as lycopene, capsanthin, bixin, and spirilloxanthin, constitute the principal pigments in particular organisms.

The overall carotenoid pattern may vary from relatively simple mixtures to extremely complex ones. The simplest mixtures may be found in foods of animal origin, owing to the limited ability of the animal to absorb, modify, and deposit carotenoids. The other extreme is the formidable array of carotenoids encountered in citrus products, dehydrated alfalfa meal, and paprika.[1,3]

The concentrations of carotenoids vary enormously from one source to another. The highest concentration of carotenes has been found in the red fringe of the corona of the pheasant's-eye narcissus, *Narcissus majalis*. Here β-carotene can constitute up to 16% of the dry weight. Furthermore, the daily rate of β-carotene formation reaches 70 μg/mg dry weight, which is more than 10,000 times the rate observed in carrot roots.[21]

The distribution of carotenoids in various plant materials has been reviewed in various publications.[1,3,22-24] Most of the data presented include those reported some years ago (generally pre-1970) using open-column chromatography and thin-layer chromatography. These methods generally do not permit adequate separation of the carotenoids, so results were usually reported as carotene, total carotene, or β-carotene. There have been rapid advances in the development of methodologies for more accurate quantitation of various carotenoids in foods in recent years, and HPLC has become widely used for these analyses.[15] This chapter includes mainly data obtained using HPLC and some aspects of the analytical conditions used by the investigators will also be presented.

Leaves and Other Vegetables

One of the earlier studies on plant materials using HPLC made use of a mixture of calcium and magnesium hydroxide for the separation of carotene stereoisomers in several vegetables, including spinach, broccoli, kale, carrot, and sweet potatoes.[25] Rather large-diameter (15-mm i.d.) columns were used, especially designed to withstand pressures up to 20 psi. The

[21] V. H. Booth, *Biochem. J.* **87**, 238 (1963).

[22] T. W. Goodwin, *in* "Chemistry and Biochemistry of Plant Pigments" (T. W. Goodwin, ed.), 2nd Ed., Vol. 1, pp. 225–261. Academic Press, New York, 1976.

[23] T. W. Goodwin, *in* "The Biochemistry of the Carotenoids," 2nd Ed., Vol. 1, pp. 96–142. Chapman and Hall, New York, 1980.

[24] T. W. Goodwin, *in* "The Biochemistry of the Carotenoids," 2nd Ed., Vol. 1, pp. 143–203. Chapman and Hall, New York, 1980.

[25] J. P. Sweeney and A. C. Marsh, *J. Assoc. Off. Anal. Chem.* 53, 937 (1970).

isomers were eluted using *p*-methylanisole in petroleum ether and acetone in petroleum ether. Fractions were concentrated and studied in a spectrophotometer. Only the percentages of the various isomers were reported.

The ease with which isomers are formed has been recognized as a problem in carotenoid analysis. Various procedures have been known to induce isomerization, including refluxing in an organic solvent, photochemical reactions, contact with active surfaces, and irradiation in the presence of iodine. Thus, inadvertent isomerization of carotenoids can take place during sample handling and analysis, e.g., during prolonged heating, exposure of solutions at room temperature to sunlight or artificial light, or even during contact with various active surfaces such as column chromatography adsorbents. The formation of cis isomers of carotenoids during the analysis of vegetables has been observed by several investigators.[25-29]

Carotenoids in leafy vegetables have been reported by several other investigators. The following studies are all carried out using reversed-phase HPLC C_{18} columns from various manufacturers. Using a mixture of methanol, acetonitrile, chloroform, and water (200:250:90:11, v/v), β-carotene and total carotenoid concentrations of 20 Thai vegetables were reported and vitamin A activity calculated based on β-carotene.[30] In the green leafy vegetables studied, β-carotene concentration ranged from 600 to 7800 μg/100 g of sample and made up 3 to 30% of the total carotenoid concentration. In a subsequent study,[31] a larger series of vegetables (50) were analyzed, and β-carotene and total carotenoid concentrations were reported. The effect of various food-processing procedures on the levels of carotenoids was also studied. β-Carotene concentrations of the leafy vegetables ranged from low levels of less than 2000 μg/100 g, e.g., in pickled mustard leaves and Asian pennywort, to high levels of about 20,000 μg/100 g for spinach and coriander, to very high concentrations of over 30,000 μg/100 g in common fennel and bitter melon leaves (Table I). The percentage of β-carotene was reported to range from 2 to 20% of total carotenoids in the vegetables.

Tuberous vegetables and beans were found to have considerably lower carotenoid concentrations. β-Carotene content was reported to range from 40 μg/100 g (cauliflower) to about 2000 μg/100 g of onion plant and garden peas.

[26] I. Stewart and T. A. Wheaton, *J. Chromatogr.* **55**, 325 (1971).
[27] S. J. Schwartz and M. Patroni-Killam, *J. Agric. Food Chem.* **33**, 1160 (1985).
[28] L. A. Chandler and S. J. Schwartz, *J. Food Sci.* **52**, 669 (1987).
[29] P. W. Simon and X. Y. Wolff, *J. Agric. Food Chem.* **35**, 1017 (1987).
[30] A. J. Speek, S. Speek-Salchua, and W. H. P. Schreurs, *Food Chem.* **27**, 245 (1988).
[31] A. J. Speek, C. R. Temalilwa, and J. Schrijver, *Food Chem.* **19**, 65 (1986).

TABLE I
LUTEIN AND β-CAROTENE CONCENTRATIONS[a] IN GREEN VEGETABLES

Vegetable	Range/mean carotenoid concentration		Ref.
	Lutein	β-Carotene	
Green, leafy (4 types)	—	330–5,030	34
Green, nonleafy (6 types)	—	217–763	
"Cruciferous" vegetables (5 types)	280–34,200	80–14,600	32
Leafy vegetables (32 types)	—	1,000–44,400	31
Tuberous vegetables and beans (16 types)	—	40–1,700	
Green, leafy (7 types)	250–10,200	1,000–5,600	35
Other vegetables (19 types)	trace–440	11–430	
Green, leafy (27 types)	73–29,900	97–13,600	36
Green, nonleafy (8 types)	142–460	74–569	

[a] Data given in micrograms per 100 g fresh weight.

In a study of five vegetables, mainly of the genus *Brassica*, three solvent systems were employed to effect the separation of several carotenoids and chlorophylls.[32] A combination of isocratic and gradient chromatography and various mixtures of methanol, acetonitrile, dichloromethane, and hexane were used. Three classes of compounds were separated: (1) xanthophylls, (2) chlorophylls and their derivatives, and (3) the hydrocarbon carotenoids. Lutein was the most abundant xanthophyll, and was accompanied by three minor cis isomers. The other two major xanthophylls were neoxanthin and violaxanthin. The only hydrocarbon carotenoids present were all-*trans*-β-carotene and its 15,15′-cis isomer. The lutein and β-carotene (the latter includes the cis isomer) concentrations are tabulated in Table I.

Other investigators had also focused on the analysis of the α- and β-carotene content of vegetables and fruits. A study of nine vegetables and fruits from different supermarkets was carried out using a mobile phase of acetonitrile, methanol, and tetrahydrofuran (40 : 56 : 4, v/v).[33] In a study of carotenoids in 22 fruits and vegetables,[34] an aqueous mobile phase of acetonitrile, tetrahydrofuran, and water (85 : 12.5 : 2.5, v/v) was used. Vitamin A activity of the foods was calculated based on the α- and β-carotene and cryptoxanthin concentrations. With the exception of carrot, β-caro-

[32] F. Khachik, G. R. Beecher, and N. F. Whittaker, *J. Agric. Food Chem.* **34**, 603 (1986).
[33] R. J. Bushway, *J. Agric. Food Chem.* **34**, 409 (1986).
[34] J. L. Bureau and R. J. Bushway, *J. Food Sci.* **51**, 128 (1986).

tene contributed to 85% of the total provitamin A activity of the vegetables. β-Carotene concentration in green leafy vegetables is clearly higher than in nonleafy varieties (Table I). α-Carotene was found in low concentrations in these vegetables, whereas cryptoxanthin was not detected in any of them. Carotenoids in carrot and the fruits studied will be discussed later in this chapter. Of the foods studied, no significant differences were reported based on either locations or months of analyses.

Carotenoids in 69 types of Finnish vegetables, fruits, berries, mushrooms, and their respective products have been reported.[35] Good resolution was said to have been obtained using a mobile phase of acetonitrile, dichloromethane, and methanol (70:20:10, v/v). In addition, a combination of isocratic and gradient chromatography was also employed for the separation of carotenoids in most extracts. Lutein and β-carotene were the predominant carotenoids in the vegetables studied, and both carotenoids occurred in higher concentrations in green leafy vegetables (Table I). In most of the vegetables studied, however, α- and γ-carotenes, cryptoxanthin and lycopene were not detected.

A study of the carotenoid composition and content of 40 tropical vegetables and 14 fruits emphasized the major carotenoids that occur in sufficient quantities to contribute significantly to dietary intake.[36] A ternary mixture of acetonitrile, methanol, and ethyl acetate (88:10:2, v/v) was used to separate the carotenoids isocratically in a C_{18} column. The method gave satisfactory separation and quantitation for lutein, cryptoxanthin, lycopene, and γ-, α-, and β-carotenes. For most of the green leafy vegetables, carotenoid compositions were rather consistent. In most cases, only β-carotene and lutein were obtained. In all the vegetables studied, β-carotene was the major carotenoid found and in 20 of the green leafy vegetables studied it made up over 50% of the sum of all carotenoids quantitated. For the remaining seven samples, at least 20% of the carotenoids was β-carotene. Lutein was also detected in all the vegetables in fairly high proportions. Except for five samples, lutein made up over 25% of all the carotenoids in these vegetables. Other carotenoids were encountered infrequently.

The carotenoid compositions of the green nonleafy vegetables were similar to those for the green leafy varieties. Seven of the eight vegetables in the former group were found to have over 25% lutein and over 30% β-carotene.

In contrast to the green vegetables, the carotenoid composition of the

[35] M. I. Heinonen, V. Ollilainen, E. K. Linkola, P. T. Varo, and P. E. Koivistoinen, *J. Agric. Food Chem.* **37**, 655 (1989).

[36] E. S. Tee and C. L. Lim, *Food Chem.* **41**, 309 (1991).

fruit and root vegetables was rather different. Although β-carotene and lutein were found in all these vegetables, several other carotenoids were also determined. For example, α-carotene was detected in carrot and pumpkin, while cryptoxanthin was found in red chilli. However, lycopene was detected only in tomato, and made up about 60% of the carotenoids quantitated.

β-Carotene concentration in the 27 green leafy vegetables studied ranged from about 100 to 14,000 μg/100-g sample (Table I). Twenty-one of the vegetables had β-carotene concentrations of over 3000 μg/100 g, or 500 μg retinol equivalent (RE)/100 g, close to the Food and Agricultural Organization/World Health Organization (FAO/WHO) recommended safe level of vitamin A intake of 600 μg RE/day.[37] Lutein concentrations varied widely, ranging from about 70 to 30,000 μg/100 g of vegetable. The eight types of green nonleafy vegetables studied had much lower β-carotene concentrations (Table I), with none of them exceeding 1000 μg/100 g of vegetable.

In summary, various studies have shown that leafy vegetables contain very high carotenoid concentrations, particularly the green leafy varieties. β-Carotene and lutein are the major carotenoids, and together can account for over 80% of all carotenoids, whereas α- and γ-carotenes, cryptoxanthin, and lycopene are infrequently encountered or occur in small concentrations.

Most of the studies of green leafy and nonleafy vegetables cited above have emphasized the content of a few major carotenoids in a variety of plant materials. Several other studies carried out on a few vegetables, have reported the analysis of a large number of carotenoids. These studies frequently require the use of a number of separation techniques and more sophisticated instrumentation. In one study employing reversed-phase HPLC, chlorophylls and eight carotenoids were separated from extracts of spinach and green algae using methanol–acetonitrile–water in a linear gradient system.[38] The study cited above of several green vegetables of the genus *Brassica* is another example of the complexities encountered and the need for various analytical techniques for the identification and quantitation of these pigments.[32] Eighteen components, belonging to 3 classes of compounds (xanthophylls, chlorophylls and their derivatives, and the hydrocarbon carotenoids) were separated from the vegetable extracts. The major constituents were also separated by semipreparative TLC and HPLC and were identified by such tools as mass spectrometry (MS), nuclear

[37] FAO/WHO, "Requirements of Vitamin A, Iron, Folate and Vitamin B_{12}," FAO Food Nutr. Ser. 23, Food and Agriculture Organization, Rome, 1988.

[38] T. Braumann and L. H. Grimme, *Biochim. Biophys. Acta* 637, 8 (1981).

magnetic resonance (NMR), and UV–visible spectroscopy. Detailed studies of squash using a combination of isocratic and gradient reversed phase HPLC systems to effect the separation of some 25 carotenoids have been reported.[39,40] A study of several carotenoids and carotenol fatty acids in various dried and canned fruits was made using two C_{18} reversed-phase HPLC columns, each with a different adsorbent.[41] Various eluants composed of mixtures of acetonitrile, methanol, dichloromethane, and hexane were used for isocratic and gradient elution, and various techniques were used in the identification of carotenoids.

The separation of carotenoids from paprika (red bell pepper) using gradient elution from a C_{18} column has been reported.[42,43] In the latter study 38 carotenoids and esters, including several unidentified components, were separated. TLC was used to assist in the separation and identification of the pigments whereas a combination of TLC and reversed-phase HPLC[44] was used in another study of several carotenoids found in paprika.

Carrots (*D. carota* L.) have traditionally been an important dietary source of carotenoids and have been studied by several investigators. The following studies all made use of a C_{18} column for reversed-phase HPLC. In a study of the separation of α- and β-carotene from raw, canned, and frozen carrots,[45] a solvent mixture of acetonitrile, tetrahydrofuran, and water (85 : 12.5 : 2.5, v/v) was used (Table II). Similar results were reported in a subsequent study, using the same mobile phase (Table II), and examining carrot from various sources obtained at three different times of the year.[34]

The separation of α- and β-carotene was also effected on a Microsorb C_{18} column using an isocratic system of methanol, acetonitrile, and dichloromethane–hexane delivered from three pumps.[46] However, only total carotene concentrations were reported for carrots. In a subsequent study of various yellow/orange vegetables, the separation of several carotenoids from raw, cooked, and canned carrot was effected using a mixture of methanol, acetonitrile, and dichloromethane (22 : 55 : 23, v/v).[47] In addition to α- and β-carotenes, ζ-carotene was also reported at a concentration of about 5.5–8.5% of the total carotenoids detected (Table II).

[39] F. Khachik and G. R. Beecher, *J. Agric. Food Chem.* **36**, 929 (1988).
[40] F. Khachik, G. R. Beecher, and W. R. Lusby, *J. Agric. Food Chem.* **36**, 938 (1988).
[41] F. Khachik, G. R. Beecher, and W. R. Lusby, *J. Agric. Food Chem.* **37**, 1465 (1989).
[42] C. Fisher and J. A. Kocis, *J. Agric. Food Chem.* **35**, 55 (1987).
[43] G. K. Gregory, T. S. Chen, and T. Philip, *J. Food Sci.* **52**, 1071 (1987).
[44] P. A. Biacs, H. G. Daood, A. Pavisa, and F. Hajdu, *J. Agric. Food Chem.* **37**, 350 (1989).
[45] R. J. Bushway and A. M. Wilson, *Can. Inst. Food Sci. Technol. J.* **15**, 165 (1982).
[46] F. Khachik and G. R. Beecher, *J. Chromatogr.* **346**, 237 (1985).
[47] F. Khachik and G. R. Beecher, *J. Agric. Food Chem.* **35**, 732 (1987).

TABLE II
α- AND β-CAROTENE CONCENTRATIONS[a] IN CARROTS

Carrot	Range/mean carotenoid concentration		Ref.
	α-Carotene	β-Carotene	
Raw	2,000–5,000	4,600–12,500	45
Canned	3,200–4,800	7,000–11,000	
Frozen	8,400–8,800	26,000–28,100	
Raw	3,790	7,600	34
Raw, A^+ hybrid	10,650	18,350	47
Freshly cooked, A^+ hybrid	15,000	25,650	
Canned	2,800	4,760	
Line B6273			
Lyophilized	3,400	6,000	29
Raw	3,200	5,200	
Frozen	3,100	5,100	
Line B9692			
Lyophilized	6,100	13,800	29
Raw	6,600	11,700	
Frozen	6,600	11,600	
HCM line			
Lyophilized	20,300	28,200	29
Raw	20,600	25,100	
Frozen	20,400	25,500	
Raw, 19 cultivars	2,200–4,900	4,600–10,300	48
Raw	3,410	6,770	36

[a] Data given in micrograms per 100 g fresh weight.

A more detailed study of carotenoids in several American carrot lines was reported using a mobile phase composed of acetonitrile, dichloromethane, and methanol for reversed-phase HPLC.[29] The carotenes separated were identified using column chromatography and thin-layer chromatography. Six carotenes, namely α-, β-, γ-, and ζ-carotenes, β-zeacarotene, and lycopene, were detected. The predominant carotenoid in all samples was β-carotene, accounting for 44–79% of all carotenes quantitated. Approximately 92–93% of the total carotenes was accounted for by β- and α-carotene, while ζ-carotene constituted about 2–4% of the total carotenes. The remaining 3–6% was made up of β-zeacarotene, γ-carotene, and lycopene. The α- and β-carotene content obtained for three of the carrot lines is tabulated in Table II. The report also included studies of changes in carotene content due to different treatment procedures.

An extensive study of 19 cultivars of carrot found in Finland has been reported.[48] The carotenoids were eluted isocratically using a mixture of acetonitrile, dichloromethane, and methanol (70:20:10, v/v) and α- and β-carotenes were the major carotenoids, making up an average of 28 and 57%, respectively, of all carotenes. Another 12% of the carotenes was γ-carotene, while lutein made up the remaining 2%. The ranges of α- and β-carotene content obtained are tabulated in Table II.

In a recent study of tropical vegetables and fruits, α- and β-carotenes were reported as the major carotenoids in carrot, composing over 90% of all carotenoids.[36] The ratio of α- to β-carotene was approximately 1:2.

Numerous studies on carotenoids in carrot have shown that α- and β-carotenes are the major carotenoids present in this root vegetable. Most of the data have shown quite consistently that the concentration of β-carotene is approximately twice that of α-carotene. The characteristic feature of carrot is its high concentration of α-carotene. Other minor carotenoids reported to be present, making up approximately 10% of total carotenoids, and γ- and ζ-carotene, β-zeacarotene, lutein, and lycopene.

Tomato, another orange-colored vegetable, has also been studied. Six samples of red-ripe Massachusetts greenhouse tomatoes were examined using several reversed-phase columns.[49] The best separation was obtained using a 5-μm particle size Partisil column (Whatman, Chifton, NJ) and a mobile phase consisting of 8% (v/v) chloroform in acetonitrile to elute the pigments isocratically. The carotenoids were detected at 470 nm. Lycopene and β-carotene occurred in a ratio of approximately 9:1 and their concentrations are tabulated in Table III.

A combination of TLC and HPLC was used in another study of carotenoids in tomatoes.[50] Two mobile phases were used for HPLC, namely, acetone–water (9:1, v/v) and acetonitrile–2-propanol–water (200:288:13, v/v). In overripe tomatoes, lycopene, β-carotene, and lutein constituted about 76, 12.4, and 3.5%, respectively, of all the pigments, with the remaining 3–5% consisting of several minor components. These minor components, particularly neurosporene and ζ-carotene, were found to occur in much higher concentrations in ripe tomatoes. The decrease in the concentrations of neurosporene and ζ-carotene in the overripe fruit was reported to be due to the conversion of these intermediates to lycopene and β-carotene.

Lycopene and β-carotene have also been reported by other investigators

[48] M. I. Heinonen, *J. Agric. Food Chem.* **38**, 609 (1990).
[49] M. Zakaria, K. Simpson, P. R. Brown, and A. Krstulovic, *J. Chromatogr.* **176**, 109 (1979).
[50] H. G. Daood, P. A. Biacs, A. Hoschke, M. Harkay-Vinkler, and F. Hajdu, *Acta Aliment.* **16**, 339 (1987).

TABLE III
β-CAROTENE AND LYCOPENE CONCENTRATIONS[a] IN TOMATO

Tomato	Range/mean carotenoid concentration		Ref.
	β-Carotene	Lycopene	
Raw	80.5–127	384–1,072	49
	660	3,100	35
	365	723	36
Paste	8,000	25,400	51

[a] Data given in micrograms per 100 g fresh weight.

as the major carotenoids present in tomatoes[35,36] (Table III). The concentrations of the two carotenoids, present in a ratio of approximately 2:1, made up close to 90% of total carotenoids quantitated. Lutein was detected as a minor component, comprising about 10% of the total carotenoids.[36] Lutein was also reported to comprise about 2.5% of all carotenoids, and another 4% was contributed by γ-carotene.[35]

More complicated chromatography procedures[51] have been used to detect the presence of other carotenoids in tomatoes. The separation of carotenoids from tomato paste using a combination of normal-phase open-column chromatography and HPLC was reported. Pigments were first eluted using a stepwise gradient of diethyl ether in petroleum ether and further analyzed by reversed-phase HPLC. For HPLC a photodiode array detector was used to monitor the carotenoids. The quantities of the four major carotenoids (lycopene, phytoene, β-carotene, and phytofluene) were 254, 167, 80, and 49 ppm, respectively (Table III). cis isomers of these carotenoids were also detected, and minor carotenoid components included α-carotene, lycoxanthin, and *cis*-mutatoxanthin.

Pumpkin is a common food item in many Asian communities, although not many studies of carotenoids in this vegetable have been reported. A group of Japanese investigators studied the composition and vitamin A value of the carotenoids in two varieties of pumpkin having different colors[52]: *Cucurbita moschata* is yellow fleshed, whereas *Cucurbita maxima* is of the orange-fleshed variety. The pigments were first separated by phase separation using liquid–liquid partition between hexane and 90%

[51] B. Tan, *J. Food Sci.* **53**, 954 (1988).
[52] T. Hidaka, T. Anno, and S. Nakatsu, *J. Food Biochem.* **11**, 59 (1987).

methanol. The fractions obtained were analyzed by open-column chromatography using a mixture of MgO–Hyflo Super Cel (1 : 1, w/w). Pigments were eluted from the column using different proportions of acetone in hexane. The three major carotenoids detected were lutein, β-carotene, and luteoxanthin, together making up about 75% of all the carotenoids, with concentrations of 922, 505, and 370 μg/100 g, respectively. Other carotenoids detected were, in descending order, taraxanthin, zeaxanthin, auroxanthin, β-cryptoxanthin, ζ-carotene, β-carotene 5,6-epoxide, and α-carotene. The vitamin A activity of the yellow variety was found to be higher than that of the orange variety, which had many oxygenated carotenoids.

Two other studies have also reported on the carotenoid composition of a pumpkin sample, determined by reversed-phase HPLC.[31,36] In one study,[36] total carotenoid concentration was less than 2300 μg/100-g sample, and the concentrations of lutein and α- and β-carotenes were 940, 756, and 578 μg/100 g, respectively. Cryptoxanthin, lycopene, and γ-carotene were not detected. The other study[31] reported a similar total carotenoid concentration, but the β-carotene content was much lower, at 49 μg/100-g sample.

Contrary to popular belief, the highest total carotenoid content or vitamin A activity is not found in orange-colored vegetables and fruits.[36] Instead, green leafy vegetables, including several local varieties used as ulam (vegetables consumed raw), have been found to be the richest sources of total carotenoids as well as provitamin A carotenes. The chlorophyll present in these leaves masks the carotenoids present. Several brightly colored vegetables and fruits, traditionally regarded as having "high carotene value," were found to have vitamin A activity values that were much lower than expected based on the color of the foods, because a large proportion of the carotenoid present in these foods was lycopene, a carotenoid with no vitamin A activity.

Fruits

Numerous investigators have studied the carotenoids in fruits, particularly the yellow- and orange-colored varieties. Data cited in this section were generated using reversed-phase HPLC and particular attention has been given to the few reports dealing with several fruits. Carotenoid compositions of fruits are generally more complex than those for green vegetables. Because of the numerous types of fruits, with different characteristics, there are large variations in carotenoid composition and content in these plant sources. Data for selected fruits are summarized in Table IV.

β-Carotene is found in most fruits, but the concentration varies widely. Low β-carotene concentrations of less than 100 μg/100 g fresh weight were

TABLE IV
CAROTENOID CONCENTRATIONS[a] IN FRUITS

Fruit	Range/mean carotenoid concentration				
	Lutein	Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
Banana	20–40	0	0	60–160	40–100
Berries, grapes, black currant	20–200	0	0	0–60	6–150
Mango	—	—	—	—	63–615
Orange, mandarin	20–30	7–300	—	20	25–80
Papaya, watermelon	0	450–1500	2000–5300	0	228–324
Starfruit	60	1070	0	0	28

[a] Data given in micrograms per 100 g fresh weight. Compiled from Refs. 31, and 34–36.

reported for various types of berries, grapes, black and red currant, and several citrus fruits.[34,35] Watermelon and plum were found to have the highest β-carotene concentrations, approximately 200–400 μg/100 g of fruit.

Oranges were found to have a low β-carotene concentration of less than 50 μg/100 g.[34–36] Orange juice concentrates have been reported to have a higher β-carotene content, ranging from 80 to 260 μg/100 g of juice.[53]

In the study of 14 tropical fruits, β-carotene concentration was found to vary widely.[36] Fruits with low levels (less than 100 μg/100 g fruit) of this carotenoid included bananas, jackfruit (*Artocarpus heterophyllus*), musk lime (*Citrus microcarpa*), and starfruit (*Averrhoa carambola*). Watermelon, papaya, papaya exotica, tree tomato, and a Thai variety of mango have β-carotene concentrations ranging from 200 to 600 μg/100 g. The mango had the highest concentration of β-carotene in this study[36] and almost all the carotenoids detected were β-carotene. Ripe mangoes were found to have five times more carotenoids than the unripe fruits, and β-carotene content was reported to be 63 μg/100 g of fruit.[31]

α-Carotene has been reported to occur in several fruits, including banana, orange, mandarin, berries, peaches, and cantaloupe.[34–36] The level of α-carotene is generally low with less than 100 μg/100 g of fruit. Cryptoxanthin is also found in several fruits, at low concentrations. However, in several other tropical fruits, e.g., papaya, starfruit, and tree tomato, over 1000 μg cryptoxanthin/100 g fruit has been reported.[36]

Lycopene occurs in high concentrations (2000 to 4500 μg/100 g in

[53] T. Philip and T. S. Chen, *J. Food Sci.* 53, 1703 (1988).

deep-orange or reddish-colored fruits such as papaya, watermelon, and pink grapefruit.[35,36,41] Lutein occurs more regularly in many fruits, at concentrations ranging from 12 to 450 μg/100 g of fruit.[35,36]

Besides the major carotenoids in fruits, the occurrence of various other carotenoids in apricots, peaches, cantaloupe, and pink grapefruit has also been reported.[41] A combination of isocratic and gradient reversed-phase HPLC was used to separate the three classes of carotenoids (xanthophylls, hydrocarbon carotenoids, and carotenol fatty acid esters). The carotenoids were monitored at six wavelengths using a photodiode array detector. The xanthophylls were identified as zeaxanthin and β-cryptoxanthin. The hydrocarbon carotenoids were identified as lycopene, γ-, ζ-, and β-carotene, phytofluene, and phytoene, as well as several of their cis isomers. The carotenol fatty acid esters were found to be present only in peaches.

The vitamin A activity of fruits is generally low. However, fruits contain more complex carotenoids, and several of these carotenoids may be of nutritional significance, in addition to being provitamin A compounds.

Roots and Tubers

Compared to vegetables and fruits, carotenoids in roots and tubers have gained less attention. Although generally low in carotenoids, roots and tubers could be significant sources of provitamin A carotenoids when consumed in sufficient quantities.

Sweet potato has been studied more frequently than other roots and tubers. Recent data, generated using HPLC, are tabulated in Table V. The large variation in carotenoid concentrations reported by various investigators[34,54,55] may depend on the variety of sweet potato studied, as these potatoes are known to vary considerably in color.

Few studies on carotenoids in cassava have been reported. The edible portion of the tuber is generally white, and would be expected to have a low carotenoid concentration. A study has shown that the total carotenoid concentration is less than 50 μg/100 g edible portion.[55] HPLC showed that the β-carotene content was only 20 μg/100 g, while lutein, cryptoxanthin, and lycopene were also detected in small quantities (Table V) and γ- and α-carotenes were not detected at all. In screening 654 clones in the cassava germplasm collection, it was found that several clones have different intensities of yellow color.[56] The β-carotene content of 21 of these clones was studied using the open-column chromatography method of the Association

[54] U. Singh and J. H. Bradbury, *J. Sci. Food Agric.* **45**, 87 (1988).
[55] E. S. Tee, unpublished observation (1991).
[56] S. N. Moorthy, J. S. Jos, R. B. Nair, and M. T. Sreekumari, *Food Chem.* **36**, 233 (1990).

TABLE V
β-CAROTENE AND LYCOPENE CONCENTRATIONS IN SELECTED ROOTS AND TUBERS

Root or tuber	Range/mean carotenoid concentration				Ref.
	Lutein	Cryptoxanthin	Lycopene	β-Carotene	
Sweet potato					
Different varieties	—	0	—	5–551	34
	—	—	—	1–4	54
Yellow variety	25	0	42	19	55
Orange variety	7	27	147	1140	55
Cassava					
White variety	2	3	1	20	55
Yellowish	—	—	—	40–790	56
Potato	13–60	Trace	Trace	3–40	34–36
Taro	3–31	1	1–3	2–16	55

[a] Data given in micrograms per 100 g fresh weight.

of Official Analytical Chemists (AOAC). β-Carotene content was indeed higher, and was found to range from 40 μg/100 g for the faint yellow clones to 790 μg/100 g for yellow tubers. Potatoes and taro are other root crops with low carotenoid concentrations (Table V).

Vegetable Oils

Crude oil of yellow maize (corn) is a fairly good source of the provitamin A carotenoids.[57] The carotenoid pigments in peanut oil have been studied during maturation.[58,59] Carotenoid esters in soybean and rapeseed oils have also been studied.[60] The concentration of carotenoids contained in raw rapeseed and linseed oils is about 30–40 ppm.[61] The major carotenes found in olive oil are β-carotene and lutein, which constitute about 30 to 31 ppm, respectively.[62,63] Other vegetable oils such as barley oil, sunflower seed oil, and cottonseed oil also show the presence of carot-

[57] S. S. Mikhitaryan, A. P. Nechaev, Y. I. Denisenko, and V. T. Lyubushkin, *Izv. Vyssh. Uchebn. Zaved., Pischch—Teknol.* 6, 29 (1966).
[58] H. E. Patte and A. E. Purcell, *J. Am. Oil Chem. Soc.* **46**, 629 (1969).
[59] E. P. Harold and E. P. Albert, *J. Am. Oil Chem. Soc.* **44**, 328 (1967).
[60] P. E. Froehling, G. Van Den Bosch, and H. A. Boekenoogen, *Lipids* 7, 447 (1972).
[61] J. Peredi, *Elelmiszervizsgalati Kozl.* **22**, 255 (1976).
[62] B. Stancher, F. Zonta, and P. Bogoni, *J. Micronutr. Anal.* **2**, 97 (1987).
[63] M. J. Minguez-Mosquera, and J. Garrido-Fernandez, *Grasas Aceites (Seville)* **37**, 337 (1986).

TABLE VI
TOTAL CAROTENOIDS FROM VARIOUS OIL PALM SPECIES[a]

Oil palm species[b]	Total carotenoids[c] (ppm)
E.o.	4347
E.o. × *E.g. (D)*	1846
E.o. × *E.g. (P)*	1289
E.o. × *E.g. (D)* × *E.g.(P)*	864
E.g. (P)	380
E.g. (D)	948
E.g. (T)	610

[a] From Ref. 74.
[b] *E.o., Elaeis oleifera; E.g., Elaeis guineensis; D. Dura; P, Pisifera; T, Tenera.*
[c] Total carotenoids estimated at 446 nm.

enoids.[61,64,65] However, the concentration of carotenoids in vegetable oils is generally low. Of the vegetable oils that are widely consumed, palm oil contains the highest known concentration of agro-derived carotenoids.[66,67]

Red palm oil is obtained from the fruits of the palm tree *Elaeis guineensis*, of which a large number of subspecies are known.[68,69] Palm forests grow in West Africa, but the tree is cultivated in East Africa, Java, Malaysia, and South America.[69,70] Palm oil from the Far East and from the Belgian Congo contains 500–800 ppm of carotenes, whereas that from the Ivory Coast (especially Dahomey) contains 1000–1600 ppm, but the oil yield is less.[69,71] Palm oil derived from Malaysian-planted *Tenera (T)* species has a carotenoid content of about 500–700 ppm.[72]

It was also reported that other oil palm species such as *Elaeis oleifera (Melanococca)* from South America have a total carotenoid content of

[64] A. I. Demchenko, *Izv. Vyssh. Uscheb. Zaved. Pishch. Teknol.* **5**, 18 (1969).
[65] A. L. Markmen and A. U. Umarav, *Uzb. Khim. Zh* **6**, 61 (1961).
[66] K. E. Ben and L. Brixius, *Fette Seifen* **67**, 65 (1965).
[67] B. Tan, *J. Am. Oil Chem. Soc.* **66**, 770 (1989).
[68] A. Beinayme, "Etudes sur de carotene de l'hulle de palme," Ser. Sci. 8. Inst. Rech. Thiles and Oleagineux, Paris, 1955.
[69] C. W. S. Hartley, *in* "The Oil Palm," 2nd Ed., Chs. 2 and 5. Longman, London, 1977.
[70] P. Blaizot and P. Cuvier, *J. Am. Oil Chem. Soc.* **30**, 586 (1953).
[71] J. C. Bauernfeind (ed.), "Carotenoids as Colorants and Vitamin A Precursors: Technical and Nutritional Applications." Academic Press, New York, 1981.
[72] S. H. Goh, Y. M. Choo, and A. S. H. Ong, *J. Am. Oil Chem. Soc.* **62**, 237 (1985).

TABLE VII
CAROTENOID CONTENTS OF PALM OIL EXTRACTS[a]

Palm oil extract	Carotenoid content[b] (ppm)
Crude palm oil	630–700
Crude palm olein	680–760
Crude palm stearin	380–540
Second pressed oil	1800–2400
Residual oil from fiber	4000–6000

[a] From Ref. 74.
[b] Total carotenoids estimated at 446 nm.

about 4000 ppm. The hybrid palms, a cross between the African oil palm *E. guineensis (E.g.)* and the South American oil palm *E. oleifera (E.o.)*, i.e., *E.o.* × *E.g.* hybrids, have been shown to provide several advantages including a more unsaturated oil, a lower height increment in trunk growth, and resistance to certain diseases.[69,73] They have been reported to have carotenoid content intermediate between that of *E. oleifera* and *E. guineensis*.[69,74,75] as shown in Table VI. The *Elaeis oleifera* species has a higher carotenoid concentration, being seven times higher than the present commercially planted species, *Tenera*, followed by the hybrids of *E.o.* × *E.g. (Dura)*.

A carotenoid-rich oil can also be obtained from the palm pressed fiber,[74,76] a palm oil by-product which is presently burnt as a fuel in palm oil mills. The pressed fiber was found to contain about 5–6% of residual oil with a carotenoid concentration of 4000–6000 ppm (Table VII), and the carotenoid content of the residual oil in the pressed fiber of hybrid oil palm fruits was even higher, at 6000–7000 ppm.[74,75]

Carotenoids from the commercial crude palm oil are concentrated during the extraction and fractionation process. A system of palm oil extraction based on a double-pressing technique has been implemented by several mills in Malaysia.[77] The expected advantages of double pressing over the conventional single-stage pressing are lower oil loss in fiber, higher

[73] J. Meunier, G. Valleja, and D. Boutin, *Oleagineux* **31**, 519 (1976).
[74] Y. M. Choo, S. C. Yap, A. S. H. Ong, C. K. Ooi, and S. H. Goh, *in* "Proceedings of the AOCS World Conference of Edible Fats and Oils: Basic Principle and Modern Practices" (D.R. Ericson, ed.), pp. 436–440.
[75] S. C. Yap, Y. M. Choo, C. K. Ooi, A. S. H. Ong, and S. H. Goh, *Elaeis* **3**, 369 (1991).
[76] Y. M. Choo, A. S. H. Ong, C. K. Ooi, and S. C. Yap, U.K. Patent Appl. 2212806 (1991).
[77] B. Sidek, and H. T. Lim, paper presented at the National Oil Palm/Palm Oil Conference —Current Development, October 11–15, 1988, Kuala Lumpur, Malaysia.

kernel extraction rate, less wear on the screw worm and cages, and reduction of contamination of the kernel oil in crude palm oil. More interestingly, the oil produced from the second pressing has a higher concentration of carotenoids (Table VII). This could be due to the fact that the first extraction (first pressing) in a double-pressing process is carried out at lower pressure (to avoid cracking of the nuts) and oil relatively higher in carotenoids was extracted. After removal of nuts the fiber is then subjected to higher pressure and more carotenoids are extracted out of the mesocarp, together with some residual oil from the first pressing.

Carotenoids are also being concentrated in an industrial process called fractionation.[78,79] Palm oil is a semisolid fat at ordinary room temperature; its semisolid nature is due to the presence of the solid, fully saturated triglycerides and the high melting point monooleo-glycerides and monolinoleoglycerides dispersed through the liquid dioleinglycerides and other more unsaturated glycerides. Fractionation is carried out to extend the uses of palm oil and the products obtained are the liquid oil (olein, 70–80%) and the solid fat (stearin, 20–30%). The liquid oil is used as cooking oil and the solid fraction can be used as a component of the harder frying fats, for the production of margarine, vanaspati component, or cocoa butter substitute. The carotenoid content in the palm olein (lower melting) fraction is enriched by 10–20%, as shown in Table VII.

Various analytical methods have been used for the determination of the carotenoid profile of crude palm oil. Column chromatography has been used in the earlier studies using different adsorbents[79–82] and reverse phase HPLC has been shown to have several advantages for the separation of carotenoids in the oil.[83,84] The major carotenoids present in crude palm oil are α- and β-carotenes, which constitute about 80–90% of the total carotenoid content. Other carotenoids are present as minor components, including ζ-carotene, phytofluene, phytoene, lycopene, neurosporene, γ-carotene, and δ-carotene, as well as xanthophylls such as zeaxanthin, α-carotene 5,8-epoxide, and β-carotene 5,6-epoxide[75,80,84] (see Fig. 1). The carotenoid composition of various species of palm oil is given in Table VIII.

The carotenoid profile for the oil extracted from fiber is slightly different in terms of chemical composition as compared with the carotenoid

[78] J. W. E. Coenen, *Rev. Fr. Corps Gras* **21**, 343 (1974).
[79] T. D. Tjang and J. J. Olie, *Planter* **48**, 201 (1972).
[80] A. T. Q. Jose, B. R. A. Delva, W. Esteves, and F. P. Gerhard, *Fat Sci. Technol.* **92**, 222 (1990).
[81] M. Agroud, *Oleagineux* **13**, 249 (1958).
[82] B. Tan, C. M. Grady, and A. M. Gawienowski, *J. Am. Oil Chem. Soc.* **63**, 1175 (1986).
[83] H. J. C. F. Nelis and A. P. De Leenheer, *Anal. Chem.* **55**, 270 (1983).
[84] J. H. Ng and B. Tan, *J. Chromatogr. Sci.* **26**, 463 (1988).

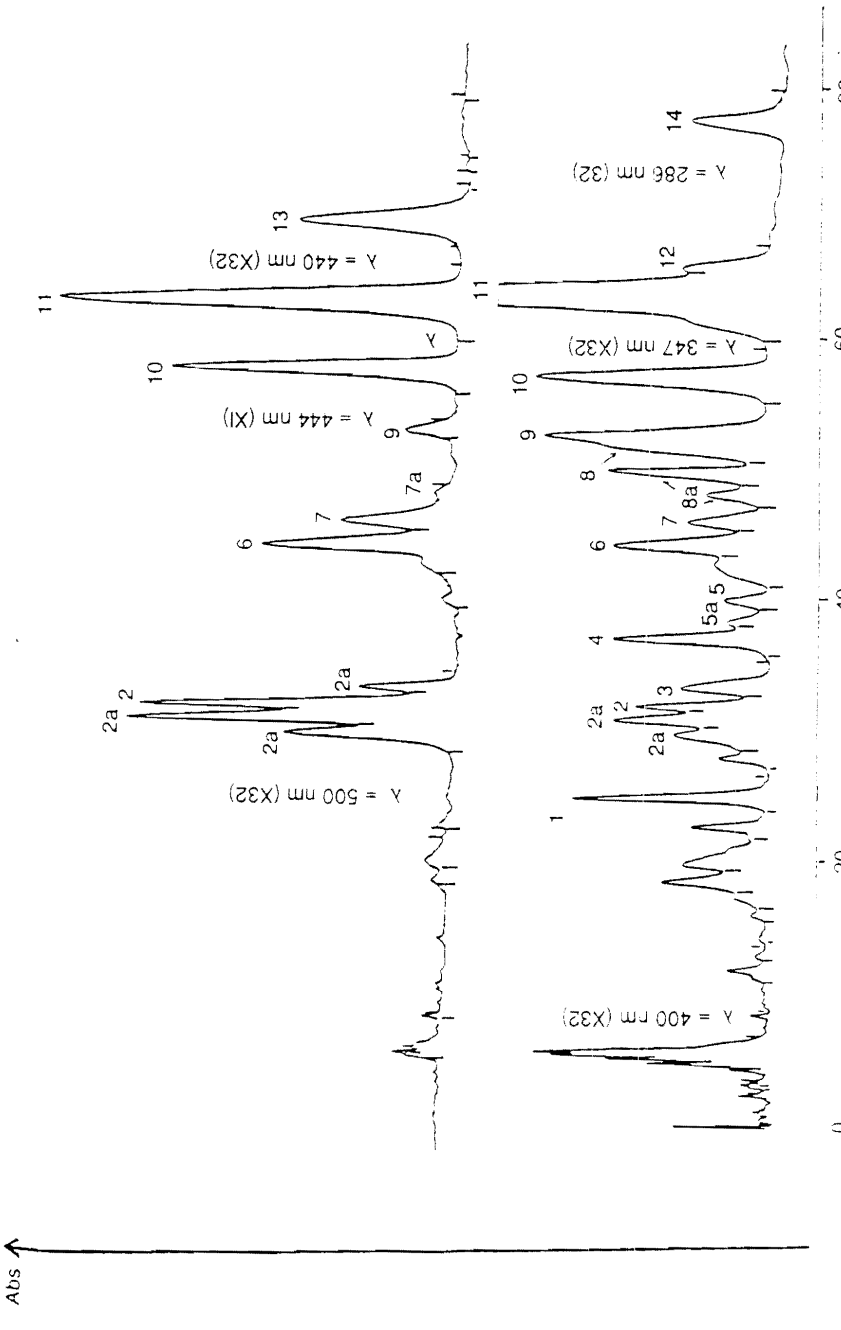

FIG. 1. HPLC of carotenoids of palm oil. Peaks: 1, presumed xanthophylls; 2, lycopene (trans and 3-cis isomers); 3, α-zeacarotene; 4, β-zeacarotene; 5, neurosporene (trans and cis isomers); 6, δ-carotene; 7, γ-carotene (trans and cis isomers); 8, ζ-carotene; 9, *cis*-α-carotene; 10, α-carotene; 11, β-carotene; 12, phytofluene; 13, *cis*-β-carotene; 14, phytoene. a, cis isomer.

TABLE VIII

PERCENT COMPOSITION OF CAROTENOIDS IN PALM OIL[a]

Carotenoids	*Elaeis guineensis (E.g.)*			*Elaeis oleifera or Melanococca (E.o.)*	Hybrids *(E.o. × E.g.)*		Palm	
	Tenera	*Pisifera (P)*	*Dura (D)*		*E.o. × E.g. (P)*	*E.o. × E.g. (D)*	Pressed fiber oil	Second pressed oil
Phytoene	1.27	1.68	2.49	1.12	1.83	2.45	11.87	6.50
Phytofluene	0.06	0.90	1.24	Trace	Trace	0.15	0.40	1.63
cis-β-Carotene	0.68	0.10	0.15	0.48	0.38	0.55	0.49	0.28
β-Carotene	56.02	54.39	56.02	54.08	60.53	56.42	30.95	31.10
α-Carotene	35.06	33.11	24.35	40.38	32.78	36.40	19.45	20.68
cis-α-Carotene	2.49	1.64	0.86	2.30	1.37	1.38	1.77	1.70
ζ-Carotene[b]	0.69	1.12	2.31	0.36	1.13	0.70	7.56	4.62
δ Carotene	0.83	0.27	2.00	0.09	0.24	0.22	6.94	2.13
γ-Carotene	0.33	0.48	1.16	0.08	0.23	0.26	2.70	2.48
Neurosporene[c]	0.29	0.63	0.77	0.04	0.23	0.08	3.38	1.88
β-Zeacarotene	0.74	0.97	0.56	0.57	1.03	0.96	0.37	0.58
α-Zeacarotene	0.23	0.21	0.30	0.43	0.35	0.40	Trace	0.15
Lycopene[d]	1.30	4.50	7.81	0.07	0.05	0.04	14.13	26.45
Total (ppm)	673	428	997	4592	1430	2324	5162	2510

[a] From Ref. 74.

[b] Together with two cis isomers.

[c] Together with a cis isomer.

[d] Together with three cis isomers.

TABLE IX
CAROTENOID CONCENTRATES FROM VARIOUS METHODS[a]

Method	Carotenoid content[b] (ppm)	Recovery (%)
Vacuum distillation[c]	>20,784	<46
Molecular distillation[c]	>80,000	>80
Adsorption[c]		
C_{18}	8,000–9,000	>90
Carbon	5,000–7,000	<50
Moleculat distillation of crude palm oil	1,290–1,990	
Adsorption from crude palm oil (activated carbon)	3,700–5,600	<80

[a] From Ref. 74.
[b] Total carotenoids estimated at 440 nm.
[c] Through methyl ester route.

profile from crude palm oil. The major carotenoids are still α- and β-carotenes, but these constitute only about 50% of the total carotenoids present. Phytoene, lycopene, ζ-carotenes, and δ-carotenes are present at higher concentrations.[74] It is interesting to note that the carotenoid profile of the second pressed oil is similar to that of the fiber oil (Table VIII).

Because carotenoids are likely to grow in importance and value, the recovery of carotenoids from palm oil and palm oil by-products is important. In addition, some studies are also being carried out to recover and concentrate carotenoids from various palm oil sources in order to obtain an oil with high concentrations of palm-based carotenoids for pharmaceutical uses. Numerous extraction methods have been developed to recover the carotenoids from crude palm oil; these include the saponification method,[85,86] urea process,[87] adsorption,[88-91] selective solvent extraction,[91,92] molecular distillation,[93] and transesterification followed by distillation of esters.[94-97]

[85] J. M. Tabor, H. F. Seibert, and P. R. Frohring, U.S. Patent 2440029 (1948).
[86] P. P. Blaizot, U.S. Patent 2652433 (1953).
[87] G. Knafo, *Bull. Mens. Inst. Technol. Etudes Recherches Gras* **6**, 323 (1952).
[88] A. S. H. Ong and P. L. Boey, British Patent 1562794 (1980).
[89] Unilever, Ltd., British Patent 691924 (1953).
[90] H. Mamuro, Y. Kubota, and H. Shiina, Japanese Patent 51282357 (1986).
[91] Y. Tanaka, I. Hama, A. Oishida, and A. Okabe, British Patent 2160874 A (1986).
[92] H. J. Passino, U.S. Patent 2615927 (1952).
[93] T. L. Ooi, A. S. H. Ong, Y. Mamuro, W. Kubota, H. Shinna, and S. J. Nakasato, *J. Jpn. Oil Chem. Soc.* **35**, 543 (1986).
[94] Lion Fat and Oil Company, British Patent 1515238 (1975).

Most of the reported methods of recovering carotenoids directly from palm oil are difficult, inefficient, or costly. Volatile palm oil methyl esters have been prepared on a large scale as an oleochemical or diesel substitute.[98,99]

A mild reaction converts palm oil (triglycerides) to volatile methyl esters, leaving the valuable minor components unchanged[100] and allowing the recovery of carotenoids in palm oil. The carotenes are concentrated or recovered from the volatile ester by adsorption,[101] solvent–solvent extraction,[96] polymer membrane,[102] and distillation.[103] The method involves selective adsorption of the carotenoids, using reversed-phase adsorption material[101]; the esters with higher polarity are eluted first from the column and then the carotenoids are recovered. The carotenoid concentrations recovered range from 8000 to 9000 ppm (Table IX). A recovery greater than 90% can be obtained using this method and the column can be regenerated and reused more than 30 times without any loss of activity. The carotenoid concentration obtained through the carbon adsorption of the crude palm methyl ester is also shown in Table IX; the recovery, as well as the carotenoid concentration, is low compared to the C_{18} reversed-phase method. Data on carotenoid content reported using the activated carbon adsorption as well as the molecular distillation of crude palm oil are also included in Table IX.

The second method involves the distillation of the volatile alkyl ester using normal vacuum distillation or molecular distillation techniques.[103] Residual concentrates of 2.0% carotenoids (Table IX) content can be achieved by normal vacuum distillation with a recovery of about 46%. This residual carotenoid can be further concentrated to 8.4% by normal-phase column chromatography and at the same time other separated minor components were also concentrated.[74] Total tocopherol and tocotrienol contents are increased to 37% and sterols are concentrated to 32%, with a recovery of 83 and 81%, respectively, based on the crude methyl ester.[100] An oil with a final carotenoid concentration of 80,000 ppm has been

[95] E. W. Eckey, U.S. Patent 2460796 (1949).
[96] N. Hara, I. Hama, H. Izumimoto, and A. Nakamura, Japanese Patent 6305074 (1988).
[97] I. Hama, Y. Tanaka, Y. Yogo, and T. Okabe, Japanese Patent 61109764 (1986).
[98] N. O. V. Sonntag, *J. Am. Oil. Chem. Soc.* **61**, 229 (1984).
[99] Y. M. Choo, A. S. H. Ong, K. Y. Cheah, and A. Baker, Australian Patent PJ 1105/88 (1988).
[100] Y. M. Choo and Ab. Gapor, *Top. Palm Oil Dev.* **14** (special issue), 39 (1990).
[101] Y. M. Choo, S. H. Goh, A. S. H. Ong, and T. S. Kam, British Patent 2218989 (1991).
[102] K. Yamada, M. Egawa, Y. Endo, and I. Hoshiga, Japan Patent 76147533 (1976).
[103] C. K. Ooi, Y. M. Choo, and A. S. H. Ong, U.S. Patent No. 5019668 (1991).

achieved through molecular distillation.[104] A carotene with a concentration of 72% has been obtained through column adsorption.[105]

A process has also been developed to produce deacidified and deodorized red palm oil from degummed palm oil with >80% of the original carotenes still intact.[106] This red palm oil is of similar excellent quality to the refined, bleached, and deodorized (rbd) palm oil that is normally traded, but contains no carotenes.

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[105] I. Hama, N. Hara, Y. Tanaka, and M. Nakamura, Japanese Patent Appl. 86/86,333 (1986).
[106] C. K. Ooi, Y. M. Choo, and A. S. H. Ong, Australian Patent PI 7267/88 (1988).

[15] Distribution of Carotenoids

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