oxidative cleavage was not mainly at the 13-position was that no significant amount of (E)-2-hexenal was found in the products. Linolenic acid is a constituent fatty acid of soybean oil, and this fatty acid will produce (E)-2-hexenal if the oxidative cleavage is at the 13-position.

The volatile constituents of bell peppers just stir fried as well as after 30 min aging at room temperature also are compared in Table 1. Decadienal and most of the volatile compounds originating from bell peppers decreased after aging, but hexanal, 2-ethylfuran, (E)-2-hexanal and (E)-2-heptenal increased. This indicates that autoxidation of unsaturated fatty acids or decadienals continued during aging. These constituents should decrease if they were not produced at the same time.

As shown in Figure 1, the heat treatment to bell peppers was very mild, and the volatile constituents of bell peppers were stable and not reactive, except those that escaped during frying or were formed during aging.

It is generally accepted that a freshly stir fried Chinese food has much better flavor quality than after it is aged. From the present work, the main change in volatile constituents of stir fried bell peppers during aging is the production of volatile carbonyl compounds from autoxidative breakdown of unsaturated fatty acids.

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Hydrocarbon Carotenoid Profiles of Palm Oil Processed Fractions

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Analysis by gradient-elution normal phase open column chromatography, thin layer chromatography and ultraviolet-visible spectroscopy (confirmation by two to three peaks), including calculation of peak ratios, tentatively revealed seven previously unreported hydrocarbon carotenoids in palm oil fractions. They were phytoene, phytofluene, ζ-carotene, α-zeacarotene, β-zeacarotene, neurosporene and ó-carotene. In addition, the presence of α-, β- and γ-carotenes and lycopene was confirmed. The carotenoid profiles of crude palm oil, crude palm olein and filtered palm olein were similar; carotenoids in these fractions totalled 700-800 ppm. Carotenoids were not found in refined, filtered and deodorized palm olein, while palm kernel oil contained 0.3 ppm of α-zeacarotene. The yield of hydrocarbon carotenoids was 30% lower in the absence of the antioxidant butylated hydroxytoluene.

In recent years, interest in the physiological function and medicinal uses of carotenoids has increased. β-Carotene appears to be synthesized and is converted to retinal in the bovine corpus luteum (1). Cantaxanthin and β-carotene, taken orally, effectively reduce the effects of exposure to the sun in patients with erythrohepatic protoporphyria, polymorphous light eruptions, and lupus erythematodes discoides (2). These two molecules also reduce the incidence of tumor formation in rats whose skin has been painted with the carcinogen benzo(a)pyrene and exposed to ultraviolet light (3).

An interesting feature of palm oil is its unusually high content of carotenoids. Some detailed analysis of the oil has been carried out (4-8), but generally, the total carotene is estimated from the absorbance at 455 nm in cyclohexane (9). The focus of this study is the elucidation of carotenoid profiles of several processed palm oil fractions by chromatographic and spectrophotometric methods.

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MATERIALS

Palm oil fractions were provided by Yee Lee Oils and Foodstuffs Co., Ipoh, Malaysia. Phytoene standard was

donated by Hoffmann-LaRoche, Inc., Nutley, New Jersey; the α-carotene, β-carotene and lycopene standards were purchased from Sigma Chemical Co., St. Louis, Missouri. Petroleum ether, diethyl ether and benzene, supplied by Fisher Scientific, Pittsburgh, Pennsylvania, were pesticide or reagent grade. Butylated hydroxytoluene (BHT) was a product of Aldrich Chemical Co., Milwaukee, Wisconsin. When protection from oxidation was desired, a concentration of 0.01% BHT was added to all solvents used for sample and column preparation and fractionation. Aluminum oxide was from Merck and Co., Rahway, New Jersey. Silica gel plates for thin layer chromatography (TLC) (0.25 mm thickness, 20 × 20 cm) were a product of Analtech, Newark, Delaware. A Buchi rotary evaporator, a clinical centrifuge and an ISCO volumeter and fraction collector were used during sample preparation and fractionation. Absorbance spectra were recorded on a Beckman Model 24 recording spectrophotometer.

METHODS

Extraction. The method was modified from Davies (10) for work with plant oils. Each palm oil fraction was placed in warm water (ca. 40 C) until the oil became a homogeneous liquid. A 1.0-ml aliquot was then drawn and 30 ml of 60% (w/v) KOH added to it. The saponification reaction was allowed to proceed 2-3 hr in the dark. In the absence of BHT, the reaction was conducted under nitrogen. The saponification mixture was extracted an average of six times with 50-ml aliquots of petroleum ether until the organic layer became colorless. The pooled organic extract was washed with 50-200 ml of distilled water, evaporated to dryness on a rotary evaporator, redissolved in 10 ml petroleum ether, and refrigerated (−10 C) overnight according to the method of Davies (10). Suspended sterols were sedimented by centrifugation at 2000 rpm for 10-20 min. The petroleum ether was evaporated from the supernatant under a stream of nitrogen to 0.6-0.7 ml for crude palm oil (CPO) and crude palm olein (CPOL) and 0.2-0.3 ml for filtered palm olein (FPOL), refined, filtered, and deodorized palm olein (RFDPOL), and palm kernel oil (PKO).

Open Column Chromatography. Normal phase open column chromatography was carried out according to the method of Davies (10). Aluminum oxide was dried for 3-4 hr at 150 C. Each column (1 × 30 cm) contained 25 g of the adsorbent, which was deactivated to Brockmann activity grade III by the addition of 4.3% (1.08 ml) distilled water, made into a slurry mixture with petroleum ether and poured into an aluminum foil-covered glass column. Chromatography proceeded in a room lit only by diffuse outdoor or indirect incandescent light.

A 0.1-ml aliquot of unsaponifiable extract was applied to the top of the column. The carotenoids were separated by 25-ml aliquots of petroleum ether containing a step-wise gradient of diethyl ether (0-20%) in petroleum ether. The flow rate was 1-2 ml/min. Fractions of 7.5 ml were collected except in the case of RFDPOL and PKO, for which 25-ml fractions were collected. When BHT was not used during elution, the column was run under nitrogen. The increased pressure increased the flow rate to 3-4 ml/min.

The absorbance spectra were scanned from 520-250 nm and wavelength maxima compared with published values (10,11) and some standards. All spectra were measured in petroleum ether unless otherwise noted. The quantity of carotenoid was calculated using the formula given by Davies (10).

Thin Layer Chromatography. The procedure of Taylor and Davies (12) was followed. Silica gel plates were heated for 30 min at 150 C prior to use. After 25 μl of standards and samples were applied to the plate, chromatograms were developed with 10% benzene in petroleum ether and visualized by spraying with 10% H_2SO_4 in ethanol and drying at 150 C for 15-30 min.

RESULTS AND DISCUSSION

Table 1 lists the hydrocarbon carotenoids identified in this study by their absorbance maxima in petroleum ether and their occurrence by palm oil fraction. Table 2 summarizes the chromatographic separation of the components of all fractions except RFDPOL and PKO. The results revealed a basic trend which led to the elucidation of the carotenoid profile: the increase in spectral wavelength and fine structure with retention time. The carotenoid profile of each palm oil fraction was determined by comparing each spectrum to published data and to this pattern.

The increase in wavelength of the maximum absorbance band, or bathochromic shift, takes place whenever the number of double bonds in the chromophore of a carotenoid increases. When chromophore double bonds are cyclized in end groups, a hypsochromic shift (decrease in the wavelength of the maximum absorbance band) occurs. Lycopene, for instance, with 11 double bonds in its chromophore, displays absorbance maxima 20-30 nm longer than those of β-carotene, two of whose 11 conjugated double bonds are in rings.

Because of this combination of desaturation and cyclization, several carotenoids can share one wavelength location. Another criterion must distinguish their spectra.

Persistence, or the degree of spectral fine structure, is this criterion (10). It is an indication of molecular structure because it depends on the vibrational transitions of the molecule. The spectra of aliphatic carotenoids show the most pronounced detail; those of monocyclic carotenoids with one double bond in the ring an intermediate amount, and those of bicyclic carotenoids with a conjugated double bond in both rings the least.

Spectra obtained from chromatographed samples displayed a bathochromic shift and an increase in persistence as retention time increased. The trend reversed itself for CPO and CPOL samples eluting after lycopene in 20% diethyl ether in petroleum ether. However, the tendency did indicate that the most retained compounds were the most unsaturated and most aliphatic ones. Carotenoids eluted, in order of increasing retention, phytoene, phytofluene, neurosporene, α-carotene, β-carotene, δ-carotene, γ-carotene and lycopene.

Compounds which eluted subsequently were probably xanthophylls. Spectra of fractions #11 and #19 from

TABLE 1

Spectral Identification of Palm Oil Carotenoids Following Open Column Chromatography

Carotenoid[a]	Absorbance Maxima (nm)[b]	Occurrence[c]
Phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene)	270-4, 282-6, 297	CPOL
Phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene)	329, 347, 367	CPOL
ξ-Carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene)	377, 400, 425	CPOL
Neurosporene (7,8-dihydro-ψ,ψ-carotene)	411-20, 432-40, 460-7	CPOL, CPO, FPOL
α-Zeacarotene (7′,8′-dihydro-ε,ψ-carotene)	391-2, 413-19, 440-3	PKO
β-Zeacarotene (7′,8′-dihydro-β,ψ-carotene)	400, 425, 451	CPOL
Lycopene (ψ,ψ-carotene)	442, 468, 500	CPO
Neo-A	438-42, 465-9, 494-8	CPOL, FPOL
γ-Carotene (β,ψ-carotene)	435-43, 460-66, 488-93	CPO, CPOL, FPOL
δ-Carotene [(6R)-ε,ψ-carotene]	432-4, 453-8, 479-85	CPO, CPOL
β-Carotene (β,β-carotene)	420-9, 442-51, 468-80	CPO, CPOL, FPOL
α-Carotene [(6′R)-β,ε-carotene]	419-20, 439-44, 464-72	CPO, CPOL, FPOL

[a]Semi-systematic names of carotenoids follow in parentheses.
[b]Wavelengths of absorbance maxima found in this study.
[c]CPO, crude palm oil; CPOL, crude palm olein; FPOL, filtered palm olein; PKO, palm kernel oil.

CPOL chromatographed in the absence of BHT and #12 and #20 from CPO shared the same position of absorbance maxima (420-3, 441-2, 467-9 nm). The spectrum of #11 was shaped like that of #12, and the spectrum of #19 like that of #20. The spectra of the former pair, however, did not resemble those of the latter pair. The early-eluting compounds had different R_f's on TLC than the late-eluting ones. Fraction #12 of CPOL with BHT gave a weak spot, separated from the origin, with an R_f of 0.11. Fraction #19 gave a very dark spot, which streaked up from the origin, with an R_f of 0.08. Although the sample spot for fraction #11 of CPO was not visible, that of #20 was a dark streak from the origin with an R_f of 0.03. The late-eluting compounds, because of their similar spectroscopic and chromatographic behavior, were probably the same xanthophyll.

The results reported here agreed with published elution sequences for carotenoids in plant and animal samples (9,13). Davies and Rees (13) state that the elution sequence of all-*trans* carotenoids from an aluminum oxide column is phytoene, phytofluene, β-carotene, β-zeacarotene,γ-carotene, neurosporene and lycopene. DeRitter and Purcell (9) give the order as phytoene, α-carotene, phytofluene, β-carotene, ξ-carotene and sterols, γ-carotene, neurosporene and lycopene.

These sequences are based on carotenoid structure. In general, the chromatographic capacity factor increases as the number of double bonds increases, but the presence of one or more rings causes a retention reversion (10). In the latter sequence, the effect of cyclization is so strong that a bicyclic compound with 10 conjugated double bonds, α-carotene, precedes an aliphatic compound with five conjugated double bonds, phytofluene.

The carotenoids in this study, with the exception of neurosporene, eluted according to these principles. This carotenoid was both the first and penultimate carotenoid eluate in FPOL. However, the published data apply to all-*trans* compounds. Analysis of absorbance spectra revealed that neurosporene which eluted early in the sequence had isomerized to the *cis* form. The ratio of the long wavelength peak (third carotenoid peak, III) to the main absorbance band (second carotenoid peak, II) expressed in percentage (%III/II) and the ratio of the ultraviolet-region "*cis* peak" to the main band ($\%D_B/D_{II}$) were used to detect the *cis-trans* conformation of

neurosporene (14). A %III/II of at least 80 and a $\%D_{II}/D_{II}$ of at least 38 for lycopene indicate the presence of the *trans* isomer. The isomerization of neurosporene, because it eluted at both the beginning (*cis* isomer) and the end (*trans* isomer) of the sequence, was most graphic in the case of FPOL (Table 2). The %III/II and $\%D_{II}/D_{II}$ for the less retained isomer of neurosporene were 74.3 and 42.9, respectively. For the more retained isomer the %III/II was 83.8; it had no "*cis* peak". The *cis* character of the former compound was emphasized further by the position of its maxima at 411, 442, 460 nm instead of at 414, 439, 467 nm. A hypsochromic shift of 2-5 nm occurs for each *cis* double bond in a carotenoid. The elution position of this compound also was in accord with the theory of Zechmeister (15) that a poly-*cis* isomer should elute far ahead of its parent all-*trans* compound.

Chromatographic and spectroscopic data of palm oil agreed well with the work of Hunter in the early to mid-1940s (4-8). Typically, liquid chromatography on active aluminum oxide of his petroleum ether-soluble and unsaponifiable matter gave, in order of increasing retention, a yellow "pre-carotene" zone, α- and β-carotenes, neo-γ-carotene, γ-carotene, neolycopene, lycopene and a brownish zone with an indefinite absorbance spectrum (4). The findings of this study confirmed the presence of α-, β- γ-carotenes and lycopene in palm oil fractions. They also revealed the presence of six other compounds for which Hunter's work gives indirect evidence.

Phytoene and phytofluene could be the colorless, non-sterol, non-saponifiable impurity present in one of Hunter's α- and β-carotene fractions (4). Two compounds, uncovered by rechromatography of fractions #9-14 from CPOL chromatographed in the presence of BHT, ζ-carotene and β-zeacarotene, could be components of the "pre-carotene" zone because of similarities in the spectral data (5,6). Spectral data in carbon disulfide which Hunter lists for neo-γ-carotene (455-62, 488-91.5, 520-6 nm) (4,5,8) agree with that for δ-carotene in that solvent (467, 490, 526 nm) (10). Thus, Hunter's work gives at least indirect support for all carotenoid components present in the palm oil samples analyzed in our laboratory.

The carotenoid profiles of CPO, CPOL and FPOL were similar to each other but markedly different from those of PKO and RFDPOL. Total carotenoid in CPO, CPOL and FPOL ranged from 700-800 ppm, and the constituents varied only slightly. All three fractions contained the core group of compounds neurosporene, α-, β-, γ-carotenes and lycopene. Neither the quantity nor content of carotenoids depended on the degree of refining. The absence of hydrocarbon carotenoids from RFDPOL, in contrast, was attributed to the rigorous refining process this fraction had undergone. PKO contained only 0.3 ppm of α-zeacarotene, which eluted in the fourth and fifth of five 25-ml fractions in petroleum ether containing 5-20% diethyl ether. The cream-colored PKO arises from the endosperm (inner kernel) of the palm fruit; the other four fractions arise from the orange-red, fleshy mesocarp. This unique carotenoid, α-zeacarotene, was found only within the endocarp (kernel).

The addition of BHT to the solvents more effectively protected the yield of hydrocarbon carotenoid than did the use of nitrogen during saponification and chromatography. Table 3 illustrates these effects by comparing the amount of some individual and total carotenoids in CPOL analyzed with and without BHT. Total carotenoids in the absence of this antioxidant dropped 31% from the total with the protection. The predominant carotenoid, β-carotene, comprised more than 80% of total carotene regardless of whether BHT was used. The amount of presumed xanthophylls (oxygenated carotenoids) increased slightly when BHT was not used, but the drop in the amount of hydrocarbon carotenoid caused the proportion of xanthophylls to approximately double. The increase in the amount of xanthophylls in the absence of BHT may be due to the oxidation of the hydrocarbon carotenoids. More constituents were separated when BHT was used. The column without BHT ran under nitrogen, however. The increase in flow rate (from 1-2 ml/min to 3-4 ml/min) and consequent decrease in resolution caused by the increase in pressure could, in part, have been responsible for the poorer separation. CPO and CPOL chromatographed in the presence of BHT gave the same number of, but different,

TABLE 2

Quantities of Hydrocarbon Carotenoids in Processed Fractions

Carotenoid	Fraction (quantity, ppm)			
	CPO + BHT	CPOL + BHT	CPOL −BHT	FPOL + BHT
Phytoene			10.1	
Phytofluene		0.3		
α-Carotene[a]				109.4
β-Carotene[b]	736.4	13.1	388.0	581.2
δ-Carotene	15.7	9.9	16.6	37.6
γ-Carotene	15.2	14.2	2.9	38.8
Neurosporene[c,d]	2.4	599.0		7.0
Lycopene	8.5			
Neo-A		23.8	18.2	
Presumed Xanthophylls	35.7	33.6	42.4	

[a]Found in other fractions with β-carotene.
[b]Included some α-carotene.
[c]Rechromatography of Fractions #9-14 of CPOL + BHT revealed that the fraction contained 20.4% neurosporene.
[d]Total quantity includes both *cis* and *trans* isomers when both were present.

TABLE 3

Effect of BHT on Carotenoid Levels in CPOL Extracts

Carotenoid	BHT (presence)[a]	BHT (absence)[a]
Phytoene[b]		10 (2.1)
β-Carotene	600 (86.5)	390 (81.6)
Xanthophylls	34 (4.9)	42 (8.8)
Others	60 (8.6)	38 (7.5)
Total	694	478

[a]Quantity of carotenoid (ppm) followed by percentage of total carotenoids in parentheses.
[b]Measurement was interfered with by BHT.

hydrocarbon carotenoids.

In this paper we have separated and identified 11 hydrocarbon carotenoids, including seven not previously isolated, in palm oil processed fractions. The increasing interest in the medical applications of this class of compounds makes their isolation and characterization crucial. Palm oil, one of the world's most widely consumed vegetable oils and a proven non-toxic food (16), could become an important commercial source of carotenoids. Future work will focus on high pressure liquid chromatographic analysis of these compounds and their potential anti-carcinogenic and/or antioxidative effects.

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Effect of Culture Conditions on Fatty Acid Composition in Lipids Produced by the Yeast *Cryptococcus albidus* var. *albidus*

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The influence of culture conditions on the fatty acid composition in lipids produced by *Cryptococcus albidus* var. *albidus* CBS 4517 was studied.

The major fatty acids in *C. albidus* var. *albidus* were oleic (18:1), linoleic (18:2) and palmitic (16:0) acid. The relative amounts of fatty acids produced varied considerably during growth and lipid accumulation phases in nitrogen-limited as well as excess-nitrogen cultures. The degree of unsaturation correlated to the lipid content in the biomass and decreased with increasing amounts of cellular lipid. After glucose exhaustion, no further changes in the fatty acid composition nor in the lipid content of the cells were observed. A number of carbon and nitrogen sources could be utilized for lipid synthesis, but they influenced the fatty acid composition only to a minor extent.

Lipid production from microorganisms provides ample opportunity for research activities, and the subject recently has been reviewed by Ratledge (1). A great deal of the work has been performed on lipid-accumulating yeast strains because a number of them are capable of accumulating large amounts of intracellular lipids (50-70%, w/w) (2,3). The lipid fraction usually has a great similarity to plant oils (1,4), and with the aim of producing substitutes for some of the more expensive types, it is important to gain increased understanding and control over fatty acid biosynthesis in microorganisms.

The yeast *Cryptococcus terricolus* Pedersen (now re-named *C. albidus* var. *albidus* (5) has been reported to have a high lipid-accumulating capacity, which is not dependent on a high C/N ratio in the medium (2,6). This is a unique characteristic because lipid accumulation in oleaginous yeasts is regarded as a two-stage process where lipid starts to be produced from excess carbon after exhaustion of nitrogen (or other nutrient except carbon) from the medium. Despite its interesting properties as a lipid producer, very little work has been reported on this strain of *C. albidus* in recent years. Boulton and Ratledge (6) compare their results concerning the constitutive lipid-producing capacity with those of Pedersen (2,7), whereas Krylova et al. (8,9) deal with lipid synthesis in *C. albidus* var. *aerius* IBFM y-229 using ethanol as the carbon source. Results from

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