Carotenoid Pigments of Peanut Oil[1]

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Abstract

A method for analysis of carotenoid pigments in peanut oil is described. The major carotenoid pigments found in peanut oil were beta-carotene and lutein. A sample of oil from immature peanuts contained 60 μg of beta-carotene and 138 μg of lutein per liter of oil. The total carotenoid concentration in oil from mature peanuts appears to be less than 1 μg per liter of oil.

Introduction

THE COLOR OF PEANUT OIL has been shown to be highly correlated with peanut maturity (1). On the basis of spectral absorption, the presence of carotenes (2) and xanthophylls (3) has been postulated. Little, if any, work has been reported concerning the qualitative and quantitative aspects of the individual pigments which give rise to the color of peanut oil. Since the nature of the carotenoids and changes which occur during maturation might help to define maturation, a study of the individual pigments was undertaken. The major carotenoids and some of those present in trace amounts have been identified.

Experimental

Plant Material

Sound, large-seeded Virginia-type peanuts were used in this study. They were separated into two maturity classes by screening. Those passing through an 18/64-in. screen were considered immature, and those riding a 25/64-in. screen were considered mature. Oil was pressed out with a Carver press.

Extraction of Carotenoids

The peanut oil, 1500 ml, was dissolved in 3 liters of 95% ethyl alcohol and refluxed with 300 g of potassium hydroxide for 2 hr. The mixture was then cooled below the boiling point of ethyl ether (30C) and mixed with 6 liters of ethyl ether. Separatory funnels were half filled with this mixture, and an equal volume of water was added with gentle swirling. After the formation of distanct phases the aqueous phase was withdrawn and gently shaken with ½ volume of ethyl ether. One volume of water was then added, and the mixture was allowed to stand approximately 20 min before the aqueous phase was withdrawn and discarded. By this procedure the last aqueous phase contained less than 20% ethyl alcohol. The most oxygenated xanthophylls would have partitioned quantitatively into ethyl ether from this mixture. The ether fraction was evaporated to approximately 12 ml of oil, which was dissolved in 20 ml of acetone and chilled overnight at −20C. The precipitate, mostly sterols, was removed by filtering through a Whatman No. 1 filter paper. Remaining color in the paper and the precipitate were removed by repeated washing with small volumes of chilled acetone (Fig. 1). The filtrate was evaporated in vacuo to about 2 ml of oil and dissolved in hexane.

Partition of the hexane solution on silica gel-methanol columns (4) was attempted, but the fractions were not distinct. Chromatography on Fisher Sea Sorb 43 magnesium oxide columns did not yield any distinct bands. To remove unidentified interfering materials, the oil was dissolved in ethyl ether, dried over calcium hydride, and refluxed for 1 hr with 1 g of lithium aluminum hydride. The excess hydride was decomposed with acetone, and the pigments were extracted by ether after the cautious addition of water. Evaporation of the ether yielded a minute amount of waxy orange residue, which was dissolved in hexane and partitioned on silica gel-methanol columns. The fractions obtained by partitioning were chromatographed from hexane on Fisher Sea Sorb 43 magnesium oxide-Hyflo Supercel, 1:1(w:w), columns. The hydrocarbon fraction was developed with 5% acetone in hexane, the monohydroxy fractions with 1% methanol and 10% acetone in hexane, and the polyhydroxy fraction with 2% methanol and 10% acetone in hexane. When distinct bands were formed, the columns were dried and the bands were cut out. The pigments were eluted from the adsorbent with hexane-acetone-methanol mixtures.

Carotenoid epoxides are reduced by lithium aluminum hydride so an alternate procedure was necessary to check for their presence. A second sample of oil, 1438 ml, was extracted as described above. After the removal of sterols the remaining oil fraction, about 10 ml, was refluxed 2 hr in 50 ml of absolute ethanol and 9 g of sodium ethylate. After refluxing, the mixture was transferred to a separatory funnel with 100 ml of ethyl ether and 250 ml of water. When distinct phases formed, the lower phase was discarded and the upper phase dried with sodium sulfate. The ethyl ether was evaporated, and the residue, 1–2 ml, taken up in hexane, was partitioned and chromatographed as described above.

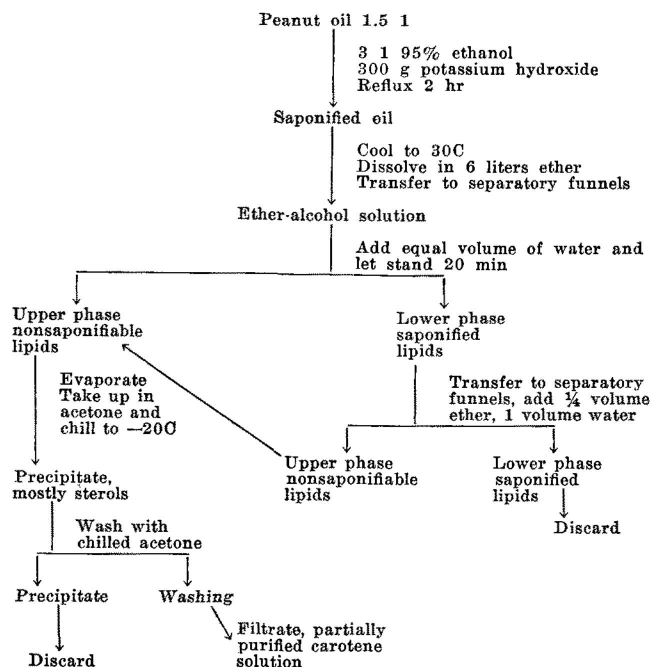

FIG. 1. Scheme for extracting carotenoids from peanut oil.

[1] Contribution from the Departments of Botany and Food Science, North Carolina State Experiment Station, Raleigh, N. C., in cooperation with the US Department of Agriculture, ARS, Market Quality Research Division, and SURDD. Paper No. 2273 of the Journal Series.
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TABLE I

Concentration of Tentatively Identified Carotenoids in Oil from Immature Peanuts

Pigment	Concentration µg per liter of oil
Alpha carotene[a]	Trace
Beta carotene	60.0
Zeta carotene[a]	0.088
Unidentified carotene	12.0
Lutein	138.0
Zeaxanthin	11.0
Flavoxanthin	5.4
Unidentified 5–8 epoxide	5.8

[a] Tentative identification.

Identification

The absorption spectra of the pigments in hexane were obtained with a Cary Model 15 spectrophotometer. Partition coefficients were obtained by the method of Petracek and Zechmeister (5). The effects of HCl in methanol on absorption spectra and on partition were determined by the method of Curl (6).

Results and Discussion

An absorption spectrum of the oil indicated carotenoid-like absorption with maxima at 480, 450, and 430 mµ. The absorption below 430 increased rapidly. A similar spectrum was obtained with the nonsaponifiable fraction after the removal of sterols.

Chromatography of the carotene hydrocarbons (lithium aluminum hydride treatment) yielded six fractions (Fig. 2). The first fraction from the column was the eluate preceding any visible or fluorescent bands. If phytoene had been present, it should have been eluted in this fraction, but none was detected by U.V. absorption between 270–300 mµ. In similar cases with other plant material, phytoene has been found by rechromatography on alumina when larger amounts of carotenes were present. The second fraction appeared on the column as a very thin, pale blue fluorescent band well ahead of the first yellow band. The chromatographic behavior was similar to phytofluene, but the spectral curve did not indicate phytofluene. The third fraction, between the fluorescent band and the first yellow band, was not visible on the column, but the eluate was slightly colored. When concentrated to 1 ml, the absorption was about 0.03 at 447 mµ. Definite conclusions cannot be drawn, but the data suggest the possibility of a trace of alpha carotene. The fourth band with the chromatographic behavior of beta carotene yielded a spectral curve identical with authentic beta carotene. The oil contained about 60 µg beta carotene per liter of oil (Table I). The next fraction was not visible on the column, but the absorption spectrum of the eluate indicated the presence of zeta-carotene, 0.088 µg per liter of oil. The last band eluted was not identified. It partitioned as a hydrocarbon, chromatographed just behind zeta carotene, and had an absorption spectrum with a shoulder at 460 mµ and maxima at 435 and 415 mµ. By using an absorption coefficient $E_{1cm}^{1\%}$ of 2500, the concentration was calculated to be 12 µg per liter of oil.

Chromatography of the hydrocarbon fraction obtained after treatment with sodium ethylate produced several bands without distinct separation. None of the fractions which were obtained appeared to contain carotenoid epoxides.

The lithium aluminum hydride monohydroxy fraction was small and, after chromatography, was found by partition coefficients to be made up mostly of lutein, which evidently leaked through the column before the main polyhydroxy band.

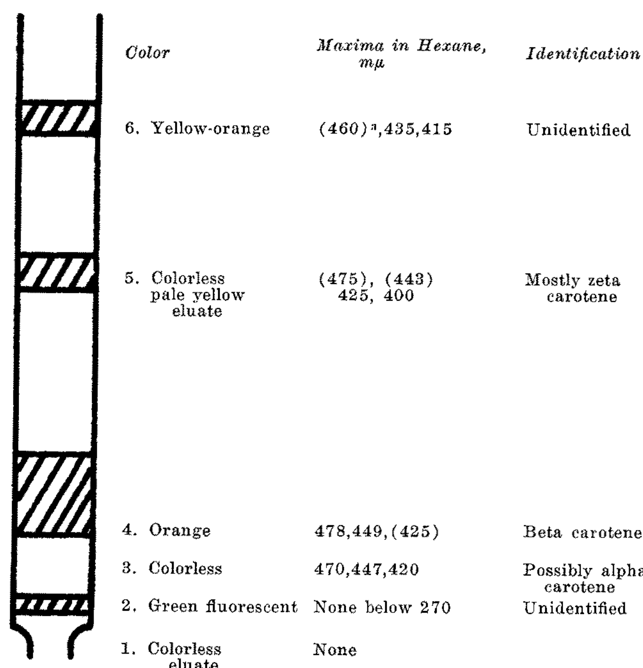


FIG. 2. Schematic chromatogram of carotene fraction from peanut oil on magnesium oxide columns after treatment with lithium aluminum hydride.

[a] Shoulders in parentheses.

Chromatography of the sodium ethylate monohydroxy fraction was most unsatisfactory. A yellow smear occupied the entire column ahead of the first distinct band. This fraction exhibited absorption maxima at 467, 438, 420, and 395 mµ in hexane. The color partitioned about 50% into 90% methanol. Addition of HCl to the methanol phase caused the 467 maxima to decrease to an indistinct shoulder. The ultraviolet spectrum indicated the possible presence of carbonyl compounds. This fraction was allowed to stand overnight in 5 ml of 80% ethanol containing 200 mg of sodium acetate and 200 mg of semicarbazide to remove the carbonyls as semicarbazones. The mixture was filtered, and the pale yellow filtrate was transferred to hexane for rechroma-

a. After treatment with lithium aluminum hydride

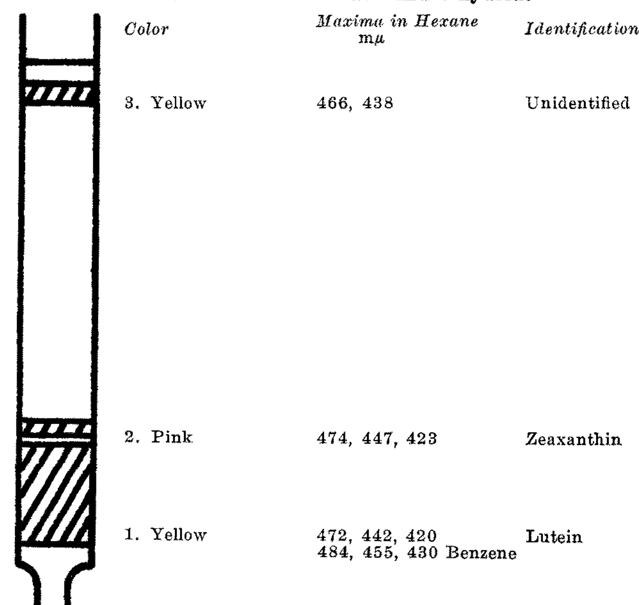
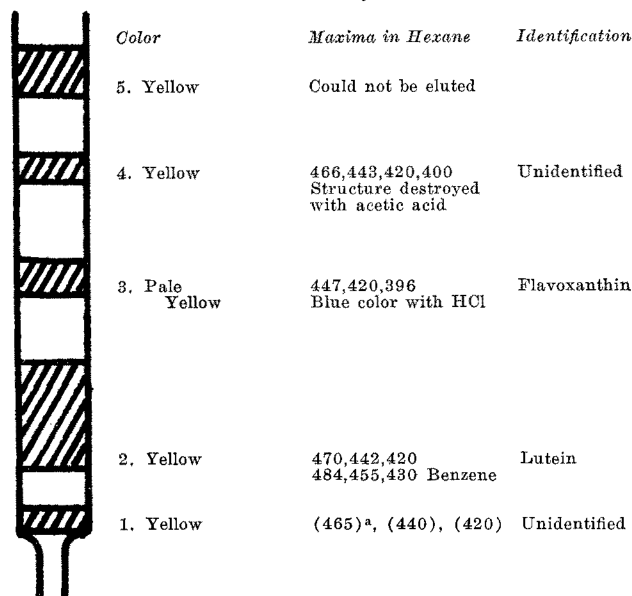


FIG. 3. Schematic chromatograms of xanthophyll fraction from peanut oil on magnesium oxide columns.

b. After treatment with sodium ethylate



[a] Shoulders in parentheses.

tography. No carotenes were found in the filtrate or in the semicarbazones after acid hydrolysis. The other fractions from the column partitioned as polyhydroxides, and none appeared to be epoxides.

Chromatography of the lithium aluminum hydride polyhydroxy fraction produced three distinct bands (Fig. 3a). The first band eluted had the chromatographic, spectral, and partition properties of lutein. When treated with HCl in methanol, the partition changed from predominantly hypophasic in a hexane-90% MeOH system to about equal distribution, indicating methylation of an allylic hydroxyl (5). The absorbance maxima shifted 1–2 mμ toward the blue end of the spectrum after treatment with the acid. The shift was probably due to formation of *cis* isomers. These data indicated that the pigment was lutein, which was the major pigment of the oil with a concentration of 138 μg per liter.

The second band was pale pink with chromatographic, spectral, and partition properties of zeaxanthin. Treatment with HCl did not change the partition properties but did change the spectrum about 1 mμ toward the blue. This pigment was assumed to be zeaxanthin present in the oil to the extent of 11 μg per liter.

The third band had chromatographic, spectral, and partion properties similar to neoxanthin. Partition and spectral properties did not change significantly with HCl in methanol. If the pigment had been neoxanthin, the acid should have converted it to neochrome with a 10–12 mμ shift in wavelength. It may be that the 5–6 epoxide was reduced to a hydroxyl by the lithium aluminum hydride treatment. Complete identification of this pigment was not attempted because of the amount available, 11 μg per liter.

Chromatography of the sodium ethylate polyhydroxy fraction again indicated lutein as the major carotenoid (Fig. 3b). The bands were less distinct on this column, and zeaxanthin was not isolated. Flavoxanthin, 5.4 μg per liter of oil, was identified. The other fraction appeared to be an unidentified 5–8 epoxy carotenoid with absorption maxima at 466, 443, 420 and 400 mμ. No evidence of neoxanthin was found. It would seem that the neoxanthin-like pigment was a product of the lithium aluminum hydride treatment.

Oil from mature peanuts appeared colorless and had no absorbance maxima in the 400–500 mμ range. No carotenes were found when 100 ml of oil from mature peanuts was extracted by the above method. It is estimated that as little as 0.1 μg of carotene could have been detected by this method so it is concluded that the oil of mature peanuts contains less than 1 μg of total carotenes per liter. This confirms the results of other workers (1–3), who also have shown that the pigments of peanut oil decrease with maturity.

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[Received September 30, 1966]