Colored Components of Processed Palm Oil

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ABSTRACT

The compounds responsible for the colors of palm oils at various stages of processing have been isolated by gel permeation chromatography. The isolated colored compounds have been characterized by visible spectroscopy and, in some cases, further fractionated by high performance liquid chromatography. All palm oils studied contained colored compounds of both higher and lower molecular weight than triglycerides. Oils at different stages of processing contained different proportions of these high and low molecular weight, colored compounds. While the color of crude palm oils was primarily due to the low molecular weight carotenoids, the color of finished oils was due to compounds other than carotenoids. High molecular weight compounds were significant, sometimes predominant, contributors to finished oil colors. These results demonstrate the importance of high molecular weight, colored compounds to the color of finished palm oils. Further, these results suggest that the difficulties associated with decolorization of certain crude palm oils may be due either to the presence of high molecular weight, colored compounds or to the presence of precursors capable of generating these compounds during the decolorization process.

INTRODUCTION

Improvements in the methods used to prepare, store and transport crude palm oils have resulted in the availability of oils of generally attractive properties (1-3). Progress toward the achievement of more efficient decolorization methods for crude palm oils has been inhibited by lack of understanding of the origin, nature and properties of compounds influencing the residual color of processed oil. While it is known that carotenoids are primarily responsible for the dark orange-red color of crude palm oil (1), the role played by these pigments in determining the residual color is not understood. Experience indicates that the quantities of carotenoids present at the time of application of decolorization processes do not, in general, determine the residual color (1,4). Of more importance to the final color are chemical changes, generally ascribed to oxidation (1,5-12), which occur subsequent to harvesting. Although unsaturated fatty acid chains (12) and possibly carotenoids (13) may be involved in this process, the mechanism by which oxidative changes affect the success of decolorization is unclear. One possibility is that carotenoids react with oxidized fatty acids to form products which are resistant to decolorization (1,12). Studies of the oxidation of methyl linoleate in the presence of β-carotene seem to support this view by showing that heating of the product mixture causes the formation of stable yellow pigments (14). Others have noted the detrimental effect on peanut oil bleaching of the presence of polymeric compounds containing oxidized fatty acid chains (15).

In an effort to elucidate the chemical reasons for the difficulties associated with palm oil decolorization, the colored compounds present in palm oils at various stages of processing were studied. This report concerns the development and use of a method for the isolation and characterization of some of the colored compounds in palm oil.

EXPERIMENTAL

Materials

Palm oils at various stages of processing were studied. In this work, "bleached oils" are oils which had been both caustic-refined and activated-clay-bleached. "Finished oils" are oils which had been processed by a series of treatments including caustic refining, activated clay bleaching and deodorization. Processing procedures employed were similar to those routinely used in the vegetable oil processing industry (16). Since the use of commercial palm oils precluded following a single crude oil through the processing stages, the samples are assumed to be representative. Finished oils had Lovibond colors in the range of 2.7-3.1 Red. In the determination of these colors, a ratio of 1 Red to 10 Yellow was maintained (17).

Unless otherwise noted, all chemicals were commercially available and were used without further purification. The methanol and chloroform used for gel permeation chromatography (GPC) were distilled and deaerated prior to use. Solvents for liquid chromatography were of HPLC grade. The GPC gel was Lipidex 5000 (Pharmacia).

Gel Permeation Chromatography (GPC)

GPC columns (2.5 x 100 cm) were prepared from 125 g of Lipidex 5000, previously swollen in the mobile phase, and wrapped in aluminum foil to prevent light exposure of the samples during chromatography. The mobile phase, 30% methanol in chloroform, was pumped at a flow rate of 1.33 mL/min in an upward direction. Samples containing about 300 mg oil/1 mL of mobile phase were introduced via a Chromatronix Teflon injection system equipped with a 1-mL loop. Progress of the separation was monitored with a PYE LCM-2 moving wire flame ionization detector and associated strip chart recorder. Components eluting before the triglycerides (TG, MW about 900) were collected in the high MW (H) fraction, those eluting after the triglycerides in the low MW (L) fraction. During and after collection, exposure to light, air or elevated temperatures was minimized. Routinely, collection times were: H, 90-165 min; TG, 165-188 min; and L, 188-360 min. Appropriate elution blanks were also collected. Fractions were either refrigerated or immediately readied for spectral analysis by rotary evaporation at temperatures below 50 C followed by dilution.

Spectrophotometric Analysis

Since the GPC fractions collected from 300 mg of finished palm oils absorbed weakly in the visible region, concentration of the solutions was necessary. Routinely, fractions were manipulated into 2 mL chloroform and the spectra recorded in self-masking microcells of 1 cm path length at appropriately sensitive settings with a Cary 219 spectrophotometer. Strongly absorbing fractions isolated from bleached or crude palm oils were manipulated into less concentrated solutions of 10 or 25 mL and the spectra were recorded in regular cells. Spectra were corrected for the absorbance of elution blanks. The magnitude of the corrections were of significance only for the weakly absorbing finished oil fractions where they were on the order of 25-40% of the observed readings. Wavelengths at which the corrections were made appear as points in Figures 2-5 and smooth curves were drawn between the points to complete the figures.

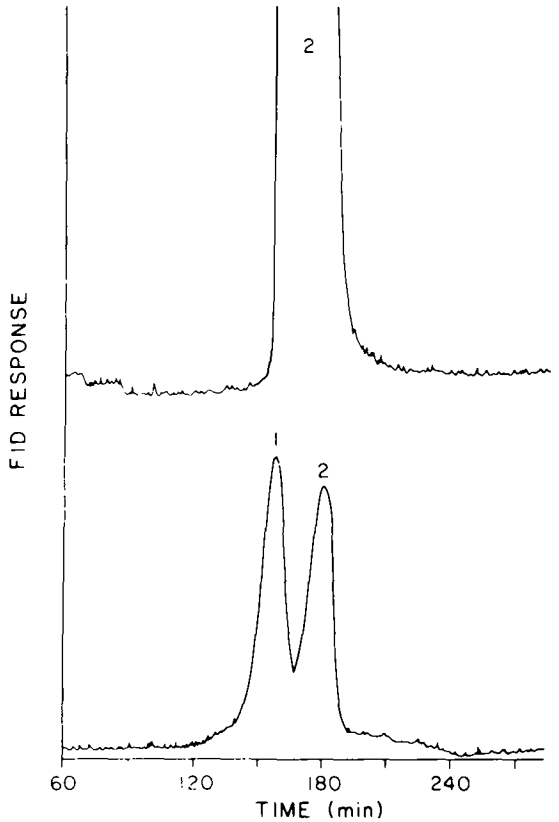

FIG. 1. Gel permeation chromatography of a finished palm oil (top curve) of Lovibond color 3.1 Red and of a sample (bottom curve) prepared by pooling H fractions from several sequential injections. Chromatographic conditions are given in the text. Peak designations are: 1) high molecular weight compounds; and 2) triglycerides.

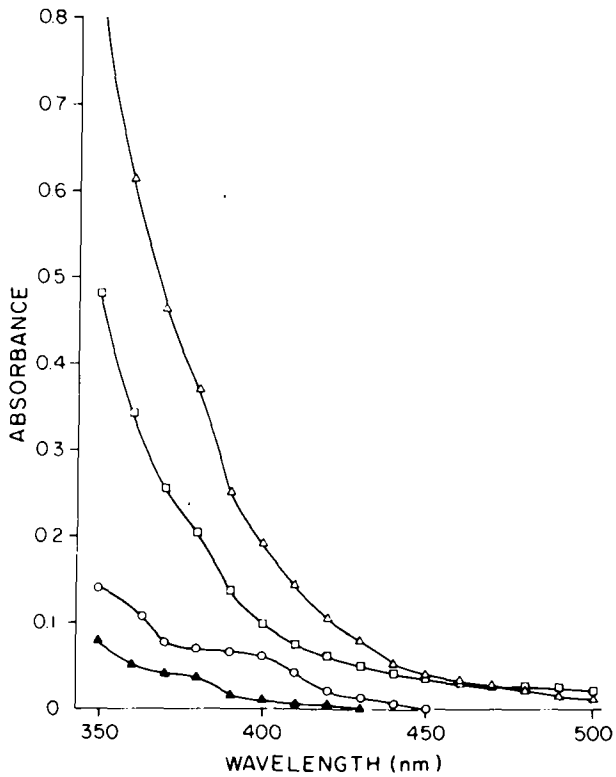

FIG. 2. Visible light absorption spectra of a finished palm oil (△) having a Lovibond color of 3.0 Red and of high (□), triglyceride (▲), and low (○) molecular weight fractions produced from it by GPC. Details of the spectral analyses for this figure as well as Figures 3-5 are given in the text.

High Performance Liquid Chromatography (HPLC)

The chromatographic system employed a DuPont Zorbax ODS (4.6 × 250 mm) column and mixtures of methylene chloride and acetonitrile as mobile phases pumped at appropriate flow rates with an Altex Model 100 pump. Spectrophotometric detection was achieved with a Schoeffel SF 770 Spectroflow spectrophotometer with GM 770 monochromator. Chromatographic parameters are given with the corresponding figures. Samples were either neat or diluted with hexane to concentrations not less than about 10%. Sample (3-20 μL) introductions were made with a Rheodyne 7120 injection system equipped with a 20-μL loop. In those cases in which comparisons between oils were made, the H fraction from 300 mg of each oil was manipulated into 50 μL of hexane solution of which 20 μL was chromatographed. Collected fractions were analyzed spectrophotometrically in a way similar to that described for finished palm oil GPC fractions.

RESULTS

Isolation of Colored Compounds by GPC

A major experimental difficulty associated with the characterization of colored compounds of finished palm oils is due to the small quantities present. Various methods for isolating these colored components from the oil triglycerides were evaluated. Saponification with refluxing methanolic potassium hydroxide resulted in an unsaponifiable fraction containing significantly more color than the starting finished palm oil. Enzymatic hydrolysis employing lipase (18-22) alone or in combination with α-chymotrypsin (23) failed to quantitatively hydrolyze the glycerides.

The limitations of the above techniques prompted an evaluation of the utility of GPC (24). Use of the Lipidex 5000 gel, similar to one used to separate numerous lipids (25), allowed an adequate separation of the components of a standard mixture. Retention times were: triglycerides (160 min), diglycerides (178 min), β-carotene (206 min), monoglycerides (224 min) and oleic acid (244 min). Under the conditions listed in Experimental, the predominant mode of separation appears to be gel permeation.

A typical GPC chromatogram of a finished palm oil (Fig. 1, top curve) shows a large triglyceride peak and a small amount of material eluting before it. A sample enriched in high MW components, when chromatographed (Fig. 1, bottom curve), illustrates the separation between high MW compounds and the triglycerides.

Visible Spectra of GPC Fractions

Visible spectra of a finished palm oil having a Lovibond color of 3.0 Red and of GPC fractions separated from it are shown in Figure 2. The spectra show the absence of absorbance bands attributable to carotenoids (λ max 456 nm for β-carotene), chlorophylls (λ max 410-440 and 670 nm [not shown]), or other known pigments. Spectra published for other deodorized vegetable oils are similar (26). Most of the components absorbing from 350-450 nm were present in the high MW (H) fraction but significant amounts were also present in the low MW (L) fraction. Relatively little light-absorbing material was contained in the triglyceride fraction.

Comparison of single wavelength absorbance readings in the 320-420 nm region indicated that the GPC technique effectively separated 80-90% of absorbing components

from the triglycerides. Since the total absorbance of the isolated fractions was only 10-20% higher than that of the original finished palm oil, the separation appears to have been performed in a manner which minimizes compositional changes among the absorbing components. This conclusion was further supported, for high MW components, by the observation that GPC of an already isolated H fraction caused no spectral change. Control experiments showed that the spectra of the isolated fractions were not appreciably affected by brief storage or by the manipulations necessary to prepare the spectral samples. The presence or absence of triglycerides did not affect the spectra of the fractions significantly.

GPC fractionation of several finished palm oils showed that the relative amounts of light-absorbing compounds in the H and L fractions were quite variable (Table I). In all cases studied, high MW components contributed significantly to the total absorbance at wavelengths between 380 and 450 nm (hence to the color) and, with the more highly colored samples, such components were the major contributors.

Crude palm oils contained a different distribution of visible-light-absorbing compounds than finished palm oils. The spectra of a crude palm oil and of the GPC fractions produced from it are shown in Figure 3. Most of the absorbing compounds were of low MW and probably were carotenoids. Similar spectra have been noted with other carotenoid-containing oils (25,26). High MW light-absorbing compounds also were present, but the H fraction spectrum contained no absorbance bands attributable to carotenoid esters or other well characterized pigments. Absorbance in the TG fraction was probably due to carotenoid pigments imperfectly separated from the triglycerides. A second crude palm oil had, in comparison to the oil of Figure 3, higher absorbance in the carotenoid region (456 nm) together with lower absorbances in its H fraction.

The effects of clay bleaching and deodorization, the major color reduction steps in vegetable oil processing, are seen in the spectra of the L and H fractions (Figs. 4 and 5, respectively). The bleached palm oil sample contained less absorbing components in its L and H fractions than crude palm oils. Deodorized palm oil contained less absorbing compounds in its L fraction and about the same (if not slightly more) in its H fraction than the bleached oil.

HPLC of the High Molecular Weight (H) Fraction

The desire to further characterize the colored compounds isolated by GPC from palm oils led to an HPLC investigation of the composition of the H fraction. Preliminary experiments indicated that fairly good separations among some of the H fraction components were obtained using a reversed-phase column and mobile phases containing 35-45% CH_2Cl_2 in CH_3CN. These experiments also showed

TABLE I

Relative Absorbance of High and Low Molecular Weight Fractions Produced from Finished Palm Oils

Color (Lovibond Red)	Relative absorbance[a]	
	H	L
2.7	0.36	0.41
3.0	0.60	0.18
3.1	0.77	0.19

[a]Values calculated from absorbance data obtained at 380 nm were normalized to that of finished palm oil. Similar values were found when data at other wavelengths in the 380-450 nm region were used.

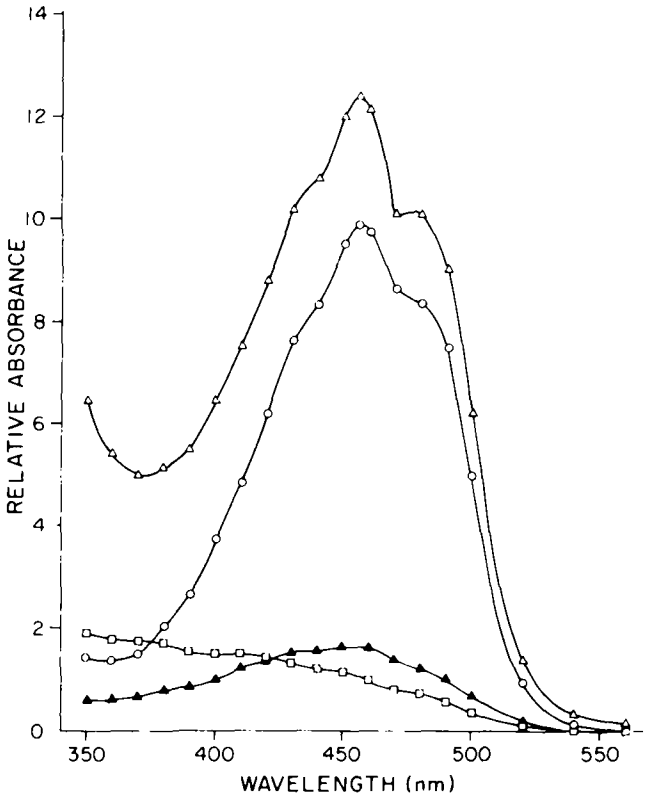

FIG. 3. Visible light absorption spectra of a crude palm oil (△) and of high (□), triglyceride (▲), and low (○) molecular weight fractions produced from it by GPC.

that it was difficult to detect, at wavelengths (380-740 nm) relevant to color, the limited quantities of components chromatographed. Consequently, the development of an adequate separation was followed using lower wavelengths where many of the components absorbed strongly. Figure 6 illustrates the complexity of an H fraction separated from a finished palm oil. While this separation was attractive, only a fraction of the light-absorbing components eluted within the chromatogram. Higher recoveries were obtained using mobile phases containing larger proportions of CH_2Cl_2, but the separations were not as good. Figure 7 shows a portion of the chromatograms obtained for finished palm oils of different colors by using 70% CH_2Cl_2 (in CH_3CN) as mobile phase. About 20% of the light- (340 nm) absorbing components eluted within the chromatographic region shown. The remainder eluted either in a region containing no peaks or in a region complicated by injection-related phenomena. The potential of HPLC is further illustrated by comparison of chromatograms obtained from palm oils at various processing stages (Fig. 8). In Figures 7 and 8, compositional differences among the light-absorbing compounds of different oils are clearly observed.

DISCUSSION

The use of GPC for the isolation of colored components from palm oil was prompted by the consideration that MW differences between triglycerides and colored compounds might exist. Among the colored compounds potentially important to palm oil color are fairly small compounds (compared to triglycerides) such as carotenes and carotene derivatives (13,27) as well as large compounds such as those formed from the condensation of carotenoids with oxidized

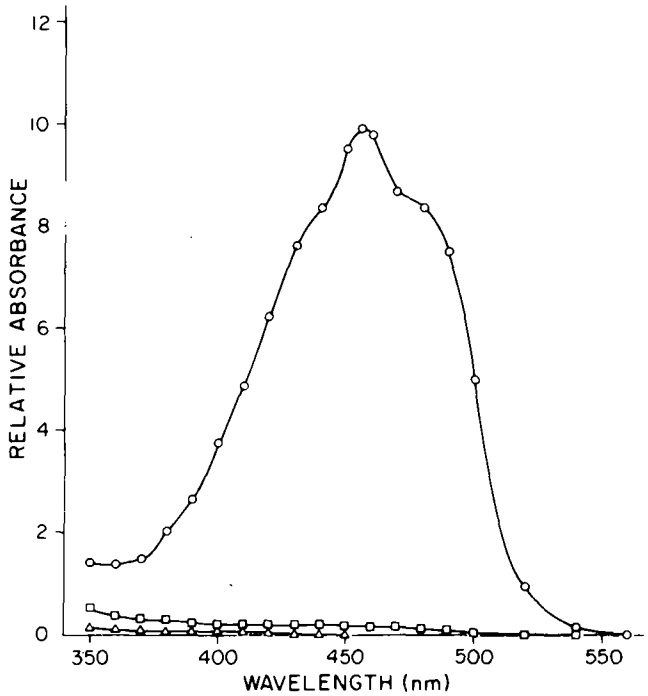

FIG. 4. Visible light absorption spectra of low molecular weight fractions produced by GPC from crude (○), bleached (□, caustic-refined and clay-bleached) and deodorized (▲, refined, bleached and deodorized) palm oils.

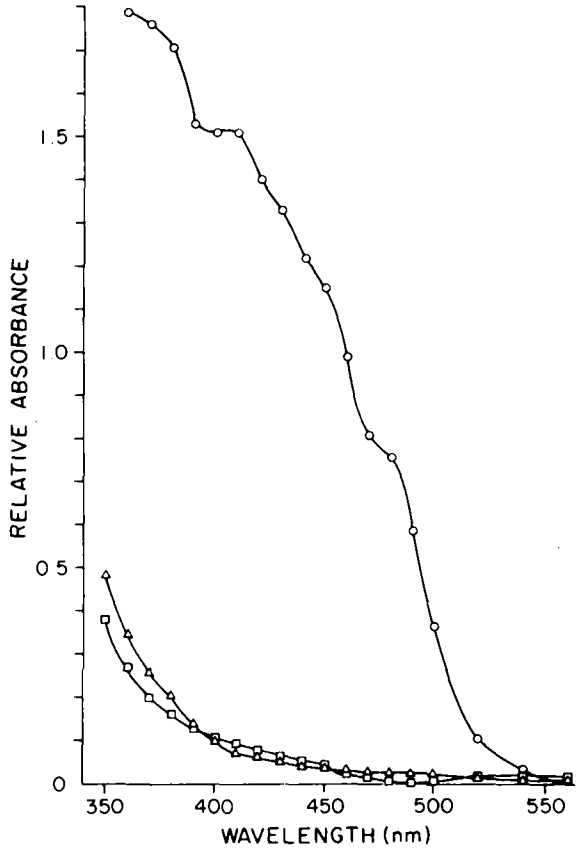

FIG. 5. Visible light absorption spectra of high molecular weight fractions produced by GPC from crude (○), bleached (□) and deodorized (▲) palm oils.

triglycerides (1). Most of the colored compounds of palm oils are indeed separable from the triglycerides by use of the GPC technique described.

Each of the palm oils studied herein contains colored compounds of higher and lower molecular weight than the triglycerides. As expected (1,2), the color of crude palm oils is primarily due to the presence of the low MW carotenoic pigments. Less expected, however, was the presence of significant amounts of high MW, colored compounds in the crude palm oils studied. It seems likely that the presence in crude palm oils of such colored compounds is a result of damage occurring subsequent to harvesting the palm fruit, however, the possibility that these compounds are natural products cannot be totally excluded. If high MW, colored compounds adversely affect decolorization and arise as a result of damage to the crude palm oil, then measurement of the quantities of these compounds might be used to predict the sensitivity to decolorization of various crude palm oils. It is worth noting that the 2 crude palm oils fractionated herein differed in the quantities of both high MW, colored compounds and carotenoids present. Since the crude palm oil containing more high MW, colored compounds also contained fewer carotenoids, it is possible that high MW, colored compounds may be derived from carotenoids. This observation is consistent with hypotheses suggesting the formation of condensation products between carotenoids and oxidized fatty acid

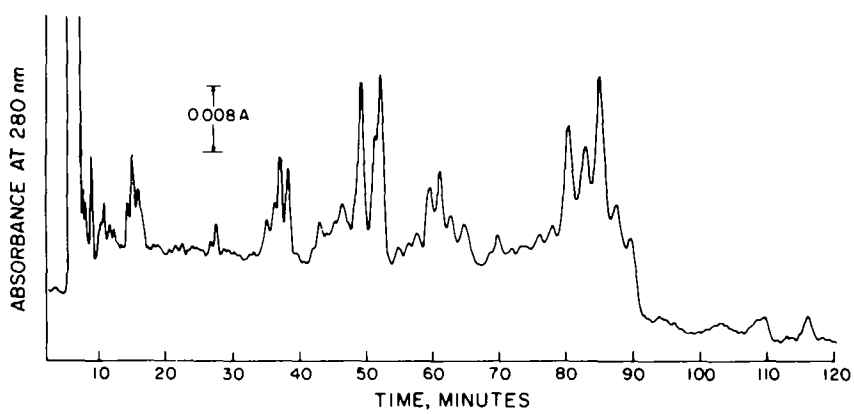

FIG. 6. Reversed-phase HPLC chromatogram of a high (H) molecular weight fraction produced by GPC from a finished palm oil of Lovibond color of 3.0 Red. Chromatographic conditions included use of a Zorbax ODS (4.6 × 250 mm) column a 37.5% CH_2Cl_2 (in CH_3CN) mobile phase, and a flow rate of 0.5 mL/min. Sample size was 3 μL of a virtually neat sample of H fraction isolated from 300 mg of oil.

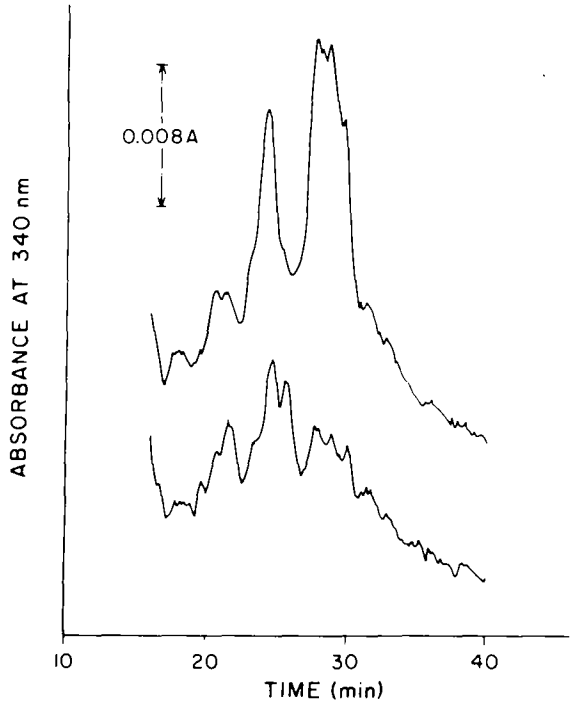

FIG. 7. Reversed phase HPLC chromatograms of H fractions produced by GPC from finished palm oils of 3.1 (top curve) and 2.7 (bottom curve) Lovibond Red colors. Chromatographic conditions were similar to those of Fig. 6 except that the mobile phase was 30% CH_3CN (in CH_2Cl_2) and the flow 0.25 mL/min.

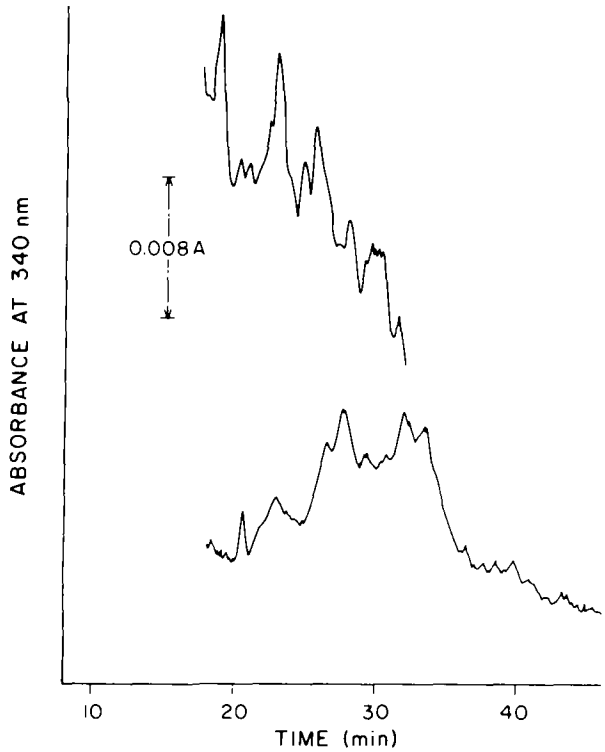

FIG. 8. Reversed-phase HPLC chromatograms of H fractions produced from a crude (top curve) and a bleached (bottom curve) palm oil. Chromatographic conditions are given in Fig. 7.

chains in triglycerides (1).

The importance of high MW compounds to the color of palm oils increases with processing. In crude oils, the contribution of high MW compounds is minimal; however, in finished oils, their contribution may predominate. This enhancement of importance of high MW, colored compounds may be due either to the fact that decolorization methods are more efficient at decolorization of low MW, colored compounds or to the fact that new, primarily high MW, colored compounds form during the decolorization process. Finished palm oils show no evidence of residual carotenoids. The detrimental role of high MW material is further illustrated by noting that more highly colored finished palm oils contained larger amounts of such compounds. On the basis of these observations, it is suggested that the reason certain crude palm oils are more difficult to decolorize may be because they have been damaged in such a way that their content either of high MW, colored compounds or precursors capable of generating high MW, colored compounds during the decolorization process is greater.

Exploratory HPLC investigations of the composition of high MW fractions isolated from palm oils have shown the extreme complexity of this fraction. Different palm oils contained different distributions of high MW components. Although the present HPLC technique is more limited than desirable, the results suggest the method may be an attractive approach to some of the central problems of palm oil color. Among these problems are the determination of the nature and origin of individual colored compounds present in crude oil and the role played by such compounds in determining the color of the finished palm oil. Furthermore, the effects on individual components of storage and processing conditions used for palm oils require elucidation. Answers to such questions could provide an improved understanding of palm oil color.

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REFERENCES

1. Loncin, M., B. Jacobsberg and G. Evrard, in "Palm Oil, a Major Tropical Product," Tropical Products Sales, Unilever House, Brussels, 1970.
2. Jacobsberg, B., Oleagineux 26:781 (1971).
3. Cornelius, J.A., Prog. Chem. Fats Other Lipids 15:5 (1977).
4. Mountjoy, P.E., and M. Pike, Presentation at AOCS 51st Annual Spring Meeting, New York, NY, 1977.
5. Jacobsberg, B., Oleagineux 30:271 (1975).
6 Jacobsberg, B., and D. Jacqmain, Ibid. 28:25 (1973).
7. Jacobsberg, B., Ibid. 30:319 (1975).
8. Johansson, G., and U. Persmark, Oil Palm News 10-11:2 (1971).
9. Ong, T.L., Rev. Fr. Corps Gras 22:27 (1975).
10. Servant, M., and Y. Bagot, Oleagineux 26:169 (1971).
11. Cornelius, J.A., JAOCS 54:943A (1977).
12. Loncin, M., and B. Jacobsberg, Ibid. 40:18 (1963).
13. Meara, M.L., and G.S.D. Weir, Riv. Ital. Sostanze Grasse 53:178 (1976).
14. Wong, K.C., in "Int. Dev. Palm Oil, Proc. Malays. Int. Symp. Palm Oil Process Mark. 1976," edited by D.A. Earp and W. Newall, Kuala Lumpur, Malaysia, publ. 1977, p. 187.
15. Lachamp, M., and M. Naudet, Rev. Fr. Corps Gras 11:205 (1964).
16. Norris, F.A., and K.F. Mattil, in "Bailey's Industrial Oil and Fat Products," 3rd Ed., edited by D. Swern, John Wiley and Sons, New York, NY, 1964.
17. AOCS Official and Tentative Methods, American Oil Chemists' Society, Champaign, IL, 1973, Method Cc 13b-45.
18. Ralston, A.W., in "Fatty Acids and Their Derivatives," John Wiley and Sons, New York, NY, 1948, p. 274.
19. Deuel, H.J., Jr., in "The Lipids," Vol. II, Wiley-Interscience, New York, NY, 1955, pp. 5-14 and 136-142.
20. Kates, M., in "Lipid Metabolism," edited by K. Bloch, John Wiley and Sons, New York, NY, 1960, p. 165.
21. Fishman, M.M., and H.F. Schiff, Anal. Chem. 48:322R (1976).
22. Tsujisawa, Y., M. Iwai and Y. Tominaga, Proc. IV IFS: Ferment. Technol. Today 315 (1972).

23. Bucolo, G., and H. David, Clin. Chem. 19:476 (1973).
24. Snyder, L.R., and J.J. Kirkland, in "Introduction to Modern Liquid Chromatography," John Wiley and Sons, New York, NY, 1974, p. 329.
25. Brooks, J.W., and R.A.B. Keates, J. Chromatogr. 44:509 (1969).
26. O'Connor, R.T., E.T. Field, M.E. Jefferson and F.G. Dollear, JAOCS 26:710 (1949).
27. Boskovic, M.A., J. Food Sci. 44:84 (1979).

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⁂High Performance Liquid Chromatography of Oxygenated Cholesterols and Related Compounds

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ABSTRACT

Twenty-four oxygenated cholesterols and structurally related compounds were analyzed by high performance liquid chromatography with silicic acid column and various mobile phases. Hexane/2-propanol was superior to hexane/tetrahydrofuran and hexane/ethyl acetate for the separation of oxygenated cholesterols. The retention volumes of oxygenated cholesterols depended on the characteristics of the substituting group, its position of substitution, as well as its orientation. The effect of various functional groups at different positions on cholesterol molecules, in general order of decreasing retention volumes, were: hydroxy on the ring, carbonyl on the ring, epoxy on the ring, hydroxy on the side chain, and carbonyl on the side chain. Synergistic effect of multiple hydroxyl substitutions on cholesterol was observed.

INTRODUCTION

In developing a quantitative method for the determination of isomeric 5α-cholestan-5,6α-epoxy-3β-ol and 5β-cholestan-5,6β-epoxy-3β-ol (1) in dehydrated eggs, we observed the superior resolution of high performance liquid chromatography (HPLC) over thin layer (TLC) and gas liquid chromatographies (GLC) for the complex mixture of cholesterol autoxidation products. HPLC simplified greatly the quantitation procedure and introduced fewer artifacts.

Among the 50 or more reported cholesterol autoxidation products (2-4), the primary stable derivatives are oxygenated cholesterols derived from substituting hydroxy, carbonyl, and/or epoxy groups on cholesterol. Recently, Ansari and Smith (5) reported the HPLC of a selected number of the cholesterol autoxidation products and demonstrated the powerful capability of HPLC for resolving isomers. In this paper, we report the HPLC of some cholesterol autoxidation products along with some structurally analogous compounds which are not necessarily found among the autoxidation products of cholesterol. The results illustrate the effect of oxygenated functional groups on retention volumes. This knowledge is useful for predicting the HPLC characteristics of related compounds and for identifying unknown compounds in the oxidation mixtures.

EXPERIMENTAL PROCEDURES

Materials

Table I lists the compounds studied by HPLC. All compounds were found chromatographically pure except 5α-cholestan-5,6α-epoxy-3β-ol which contained ca. 5% 5β-cholestan-5,6β-epoxy-3β-ol. No further purification was attempted. The solvents were glass-distilled, HPLC grade, purchased either from Burdick and Jackson, Muskegan, MI, or Fisher Scientific Co., Pittsburgh, PA.

HPLC

HPLC was carried out with a Waters Associates' instrument (Model ALC/GPC 244, Milford, MA) using μPorasil column (3.9 mm × 30 cm). Elution was monitored by an absorption detector (variable wavelength Model 450, Waters Associates) set at 210 nm and a differential refractometer (R-401, Waters Associates) connected in series. Chromatograms were recorded with a dual pen strip chart recorder (Linear Instrument Co., Irvin, CA). Refractometer signals were integrated using Auto-lab System I (Perkin Elmer, Norwalk, CN).

TABLE I

Compounds Studied by HPLC

	mp (C)	Source[a]	Remark[b]
5-Cholesten-3β-ol (Cholesterol)	147-149	1 & 2	
5α-Cholestane	78-80	1	(N)
5-Cholestene	89-90.5	1	(N)
3,5-Cholestadiene	77-79	1	(N)
5α-Cholestan-3β-ol	142-144	2	(N)
5β-Cholestan-3β-ol	101-103	2	(N)
5-Cholesten-3α-ol	142.5-143	1	(N)
5,7-Cholestadien-3β-ol	148-150	3	(N)
5,24-Cholestadien-3β-ol	118-120	1	(N)
5α-Cholestane-3β,6β-diol	–	1	(N)
5-Cholestene-3β,7α-diol	183.5-184.5	1	(O)
5-Cholestene-3β,7β-diol	178-180	1	(O)
5-Cholestene-3β,20-diol	127-128	1	(O)
5-Cholestene-3β,25-diol	178-179	1	(O)
5α-Cholestane-3β,5,6β-triol	230-234	1	(O)
5α-Cholestan-3-one	129-130	2	(O)
5-Cholesten-3-one	127.5-130	1	(O)
5,3-Cholestadien-7-one	111-113	1	(O)
5α-Cholestan-3β-ol-6-one	145-147	3	(N)
5α-Cholestan-3β-ol-7-one	161-164	1	(N)
5-Cholesten-3β-ol-7-one	174-177	1	(O)
5-Cholesten-3β-ol-22-one	–	3	(N)
5α-Cholestan-5,6α-epoxy-3β-ol	137-139	1	(O)
5β-Cholestan-5,6β-epoxy-3β-ol	132-132.5	4	(O)

[a]Sources of supply were: 1) Steraloids Inc., Wilton, NH 03086; 2) Supelco, Inc., Bellefonte, PA 16823; 3) Sigma Chemical Co., St. Louis, MO 63178; 4) synthesized according to the procedure reported by Chicaye, Powrie and Fennema (6).

[b]N: not a reported autoxidation product of cholesterol; O: a reported autoxidation product of cholesterol.