Noncarotenoid Hydrocarbons in Palm Oil and Palm Fatty Acid Distillate

S.H. Goh and **P.T. Gee**
Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia

Column chromatographic and gas chromatographic-mass spectroscopic (GCMS) analyses for minor and trace noncarotenoid hydrocarbons of crude palm oil and palm fatty acid distillate revealed the presence of a wide range of n-alkanes ($C_{12}H_{26}$ to $C_{36}H_{74}$) and n-alkenes in addition to the major component, squalene. Hydrocarbon components concentrated in palm fatty acid distillate where squalene was dominant, but degradation products such as alkenes (from fatty acids or glycerides), aromatic hydrocarbons (from carotenes) and diterpene hydrocarbons (from tocotrienols) were detected in significant quantities, superseding the naturally occurring n-alkanes. Mechanisms proposed suggest that degradation of the valuable vitamin E or tocotrienols needs to be minimized in physical refining.

Hydrocarbons are the least polar of natural compounds. They can be classified into straight-chain, branched-chain, cyclic, aromatic and terpenoid types based on their structures. Most sources of oils and fats provide small amounts of hydrocarbons. The predominant hydrocarbon component is usually squalene, an acyclic triterpene. Squalene is a colorless compound because the six double bonds in the molecule are not conjugated; its presence in various vegetable oils, including palm oil, has been documented (1-2). In crude palm oil, a group of tetraterpenes called carotenes is the most abundant and also the most important of hydrocarbon components. These carotenes constitute about 500-700 ppm of the oil (3), and their presence gives rise to the characteristic orange-red color of crude palm oil. The natural carotenes found in crude palm oil are mainly α- and β-carotene with lesser amounts of γ-carotene, lycopene and xanthophylls. It also has been reported (4) that phytoene and phytofluorene (also tetraterpenes) are present. Carotenes found in crude palm oil have very high pro-Vitamin A activity due to major amounts of β-carotene. However, during the present refining processes, all the carotenes are thermally and catalytically degraded by heat and the action of bleaching earth. During the bleaching process, small amounts of aromatic products such as toluene, xylenes, dimethylnapthalenes and ionenes were reported to be formed (5). Investigation of other polycyclic hydrocarbons revealed that only traces were detectable (6); steam distillation and adsorptive earths remove most of these trace aromatics so that their content usually is lower than in the unrefined crude palm oil.

Straight-chain hydrocarbons normally are present in oils at trace levels. In palm oil, only hentriacontane has been reported to be present in the nonsaponifiable matter (7). Other hydrocarbons reported in palm oil include an unknown sesquiterpene (8) and two C-20 hydrocarbons (7). Hydrocarbons in vegetable oils normally are analyzed on the unsaponifiable matter after removal of the bulk of glycerides by saponification using strong alkali. In this paper, the hydrocarbons of palm oil and palm free fatty acid distillate (PFAD) are studied.

EXPERIMENTAL

Materials. Palm fatty acid distillate (PFAD) was obtained from Lam Soon Oil & Soap Mfg. Sdn. Bhd., Petaling Jaya, Selangor. Crude palm oil (CPO) samples were obtained from Lam Soon (above) and Chemara Research Station, Kumpulan Guthrie Sdn. Bhd., Seremban, Negeri Sembilan. Thin layer chromatographic plates, silica gel (Merck 9380) and alumina for column chromatography were purchased from Merck (Darmstadt, Germany). Hydrocarbon standards were purchased from Sigma Chemical Co., (St. Louis, Missouri) or Applied Science (State College, Pennsylvania). Other chemicals were of analytical or reagent grades and were used without further purification.

Proton (100 MHz) and Carbon-13 (25 MHz) nuclear magnetic resonance (NMR) were recorded on a Jeol JNM-FX100 Fourier Transform NMR spectrometer in deuterochloroform solutions with tetramethylsilane as the internal standard. Chemical shifts are reported as δ ppm downfield from tetramethylsilane. Visible spectra were recorded on a Beckman DU-7 UV-VIS spectrometer in a 10 mm cell. GC was performed on a Tracor 560 equipped with a flame ionization detector (FID) using a four-ft 1/8" stainless steel column packed with 1% SE-30. High performance liquid chromatography (HPLC) was performed on a 30 × 0.39 (i.d.) cm silica gel (10u) column using a Waters Associates liquid chromatograph Series 200 with R-401 Series refractometer and Model 440 (254 nm) absorbance detector. GCMS or direct probe mass spectrometry (electron impact) were performed on a Kratos AEI MS3074 mass spectrometer with a DS55 data system.

Purification of solvents. n-Hexane was purified with concentrated sulfuric acid to remove aromatic compounds and distilled from sodium/benzophenone. Other solvents were distilled before use.

Isolation of hydrocarbons from CPO. Non-carotenoid hydrocarbons were isolated from crude palm oil by column chromatography with 150 g of silica gel (Merck 9380) packed in a 3 cm diameter column. Crude palm oil (75 g) in 150 ml of hexane was loaded into the column, and elution was by hexane. Fractions were collected until the colored carotenoids emerged. The colorless hydrocarbon fractions were combined and rotary evaporated to a small volume containing ca. 3 mg of product before analysis.

Isolation of hydrocarbons from PFAD. A pre-elimination of free fatty acid was carried out by basic alumina column chromatography. PFAD (50 g) was dissolved in a minimum amount of chloroform at room temperature and loaded into a column packed with basic alumina (100 g) in chloroform. The solvent used for elution was chloroform, and elution was continued until the free fatty acids began to emerge as indicated by the change of column material from opaque to translucent. The total eluent was rotary-evaporated and redissolved in a small volume of hexane (ca. 5 ml). Thin layer chromatography (TLC) using hexane as the solvent revealed that tocopherols, sterols, methyl esters and diglycerides were present among the noncarotenoid hydrocarbons.

Further purification of noncarotenoid hydrocarbons was achieved by silica gel column chromatography using hexane as solvent. Total hydrocarbons obtained made up 1.5 g of light yellow oil, which was then analyzed by GC, HPLC and GC-MS.

The hydrocarbon fraction was further classified into alkanes and alkenes, di- and trienes, diterpenes and squalene by rechromatography on another, similar silica gel (20 g) column. Alkanes and alkenes were eluted first (first 10 ml), followed by di- and trienes (next 15 ml), then diterpenes (next 150 ml) and finally squalene (last 200 ml).

TABLE I

Hydrocarbons in Crude Palm Oil (CPO) and Palm Fatty Acid Distillate (PFAD)

Hydrocarbon	CPO[a]	PFAD[a]	Hydrocarbon	CPO	PFAD
$C_{12}H_{26}$ n-alkane	tr	–	$C_{15}H_{30}$ n-alkene	tr	S
$C_{13}H_{28}$ n-alkane	tr	–	$C_{16}H_{32}$ n-alkene	S	tr
$C_{15}H_{32}$ n-alkane	M	tr	$C_{17}H_{34}$ n-alkene	S	tr
$C_{16}H_{34}$ n-alkane	M	–	$C_{18}H_{36}$ n-alkene	tr	M
$C_{17}H_{36}$ n-alkane	M	tr	$C_{19}H_{38}$ n-alkene	–	tr
$C_{18}H_{38}$ n-alkane	M	–	$C_{20}H_{40}$ n-alkene	tr	–
$C_{19}H_{40}$ n-alkane	M	–	$C_{21}H_{42}$ n-alkene	–	S
$C_{20}H_{42}$ n-alkane	M	–	$C_{24}H_{48}$ n-alkene	–	M
$C_{21}H_{44}$ n-alkane	M	–	$C_{26}H_{52}$ n-alkene	–	S
$C_{22}H_{46}$ n-alkane	M	–	$C_{15}H_{28}$ diene	tr	–
$C_{23}H_{48}$ n-alkane	S	–	$C_{16}H_{30}$ diene	tr	–
$C_{24}H_{50}$ n-alkane	S	–	$C_{17}H_{32}$ diene	tr	–
$C_{25}H_{52}$ n-alkane	S	–	$C_{23}H_{44}$ diene	–	S
$C_{26}H_{54}$ n-alkane	S	–	$C_{26}H_{50}$ diene	–	M
$C_{27}H_{56}$ n-alkane	S	–	$C_{18}H_{32}$ triene	tr	–
$C_{28}H_{58}$ n-alkane	S	–	$C_{19}H_{34}$ triene	S	–
$C_{29}H_{60}$ n-alkane	M	–	$C_{26}H_{48}$ triene	–	S
$C_{30}H_{62}$ n-alkane	S	–	$C_{20}H_{32}$ diterpenes	–	L
$C_{31}H_{64}$ n-alkane	M	tr	$C_{30}H_{50}$ triterpene	VL	VL
$C_{33}H_{68}$ n-alkane	M	–	C_8H_{10} aromatic[b]	O	tr
$C_{36}H_{74}$ n-alkane	M	–	$C_{12}H_{12}$ aromatic[b]	O	S
$C_{12}H_{24}$ n-alkene	S	–	$C_{13}H_{18}$ aromatic[b]	O	M
$C_{14}H_{28}$ n-alkene	M	–			

[a]Relative amounts: O, not detectable; tr, trace; S, small; M, medium; L, large; VL, very large, and blanks (–), either unresolved or not detected; VL ≅ 10L ≅ 100M ≅ 500S ≅ 2000 tr.

[b]Xylene, dimethylnaphthalene and ionene.

RESULTS AND DISCUSSION

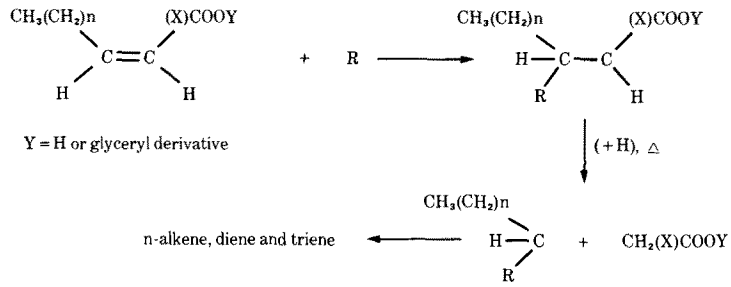
As hydrocarbons are the least polar group of compounds found in nature, they can be separated easily from other constituents by column chromatography on silica gel using an appropriate solvent for the elution. In crude palm oil, noncarotenoid hydrocarbons can be collected conveniently before the elution of the colored carotenoids if hexane is used as the eluting solvent. In palm free fatty acid distillate, the hydrocarbon fraction has to be pre-separated from the bulk of the fatty acids prior to isolation of the noncarotenoid hydrocarbons by column chromatography. Although individual classes of hydrocarbons can be isolated by further column chromatography or TLC, the noncarotenoid hydrocarbon mixture can be analyzed directly by GC or GC-MS.

In crude palm oil and fatty acid distillate the noncarotenoid hydrocarbons are not the same, either qualitatively or quantitatively, except that squalene is the major component in both cases. Results of GC-MS analysis are summarized in Table I. As seen from the table, a wide range of n-alkanes, $C_{12}H_{26}$ to $C_{36}H_{74}$ (except $C_{14}H_{30}$, $C_{32}H_{66}$, $C_{34}H_{70}$ and $C_{35}H_{72}$) were observed. Smaller amounts of alkenes ($C_{12}H_{24}$, $C_{14}H_{28}$ to $C_{18}H_{36}$ and $C_{20}H_{40}$), alkadienes ($C_{15}H_{28}$ to $C_{17}H_{32}$) and alkatrienes ($C_{18}H_{32}$ and $C_{19}H_{34}$) also were detected.

The hydrocarbons isolated from PFAD are expected to be similar to those of crude palm oil except that additional degradation products from thermal decomposition of carotenes may be obtained. However, Table I reveals that, in addition to diterpenes, many more higher olefinic hydrocarbons were obtained from PFAD. Our estimates of non-terpenoid hydrocarbons in CPO and PFAD are ca. 30-50 and 4000-8000 ppm, respectively. n-Alkanes were detectable in crude palm oil, but in PFAD most of these peaks apparently were being swamped by large amounts of alkenes and alkadienes. Electron impact GC-MS tends to favor the detection of unsaturated rather than saturated hydrocarbons. Higher alkenes constituted a relatively significant proportion of PFAD hydrocarbons with $C_{15}H_{30}$, $C_{18}H_{36}$, $C_{21}H_{42}$ and $C_{24}H_{48}$ as the major components. Small amounts of $C_{15}H_{30}$, $C_{21}H_{42}$ and $C_{26}H_{52}$ also were present. The quantity of alkadienes (mainly $C_{26}H_{48}$ and smaller amount of $C_{23}H_{44}$) was relatively higher than the triene $C_{26}H_{48}$. Alkenes from CPO include $C_{12}H_{24}$ to $C_{17}H_{34}$.

Because the longer chain alkenes ($C_{18}H_{36}$ to $C_{26}H_{52}$) were not observed in sufficiently large amounts in crude palm oil, their presence in PFAD is attributed to thermal/catalytic reactions during the refining of palm oil. Scheme 1 shows a possible mechanism of the formation of these alkenes. The proposed mechanism involves a radical addition to a double bond followed by bond cleavage resulting in the formation of these alkenes. The source of radicals is probably the decarboxylation of fatty acids by the action of trace metals.

An interesting finding in the hydrocarbons from PFAD is that it contains $C_{20}H_{32}$ compounds, i.e. four isomeric acyclic diterpenes, one of which constituted the second most abundant hydrocarbon (being less abundant only than squalene). This group of compounds was purified by silica gel column chromatography or HPLC using anhydrous hexane as the solvent. Proton NMR of the total diterpenes revealed a low field R-C*H*=CH_2 proton at 6.38 ppm (dd, J 17.2 Hz, J' 10.4 Hz, 1H), indicating that a monosubstituted ethylene group is present and forms part of a conjugated diene system. About 6-7 other olefinic protons resonate between the range of 5.00 and 5.33 ppm. Two doublets are observed in this region; one, at 5.24 ppm (J 17.3 Hz), is assigned to the *trans* proton of terminal olefin, and the other, at 5.06 ppm (J 10.5 Hz), is assigned to the *cis* proton. Overlapping with one of the *cis* proton doublets is a singlet (at δ 5.01 ppm) belonging to the terminal methylene protons of a typical disubstituted olefin. These observations suggested a partial structure of $-C(=CH_2)CH$



Fatty Acid	n	X	Radical source (fatty acid)	R	Product
Linolenic	1	$C_{13}H_{22}$	Palmitic	$C_{15}H_{31}$	$C_{18}H_{36}$
Linoleic	4	$C_{10}H_{18}$	Palmitic	$C_{15}H_{31}$	$C_{21}H_{42}$
Oleic	7	C_7H_{14}	Palmitic	$C_{15}H_{31}$	$C_{24}H_{48}$
Oleic	7	C_7H_{14}	Stearic	$C_{17}H_{35}$	$C_{26}H_{52}$
Oleic	7	C_7H_{14}	Oleic	$C_{17}H_{33}$	$C_{26}H_{50}$
Oleic	7	C_7H_{14}	Linoleic	$C_{17}H_{31}$	$C_{26}H_{48}$
Linoleic	7	$C_{10}H_{18}$	Oleic	$C_{17}H_{33}$	$C_{23}H_{44}$

SCHEME 1. Formation of n-alkene, diene and triene.

$=CH_2$. Other vinylic protons were not resolved in this region. In the high field region, a triplet at 2.23 ppm accounts for the protons allylic to the 1,3-butadiene group. At 2.02 ppm a broad singlet can be assigned to the allylic methylenic protons. Two singlets were observed at 1.60 and 1.67 ppm; these are typical of signals due to methyl protons on *trans* and *cis* substituted double bonds, respectively, in acyclic isoprenoids. Based on the proton NMR alone the major component of the acyclic diterpene can be identified as 7,11,15-trimethyl-3-methylenehexadeca-1,6,10,14-tetraene(**1**). Other small signals include the protons at ca. 2.7 ppm, methyl doublets at 0.90 ppm (J 5.9 Hz) and 1.09 ppm (J 7.8 Hz), and methylene singlet at 1.26 ppm. Also observed were olefinic signals at ca. 5.4-5.6 and ca. 7 ppm.

The ^{13}C-NMR of a purified sample of 1 (ca. 90%) also provided useful information. The ^{13}C chemical shifts of the major component show good agreement with the reported values (9) (Table II). Also, the observation of chemical shifts of the methyl carbons of C-18 and C-19 at 16.07 ppm and the methylene carbons of C-8 and C-12 at 39.75 ppm indicated that the 6,7- and 10,11- double bonds in the compound possess E-configuration. The corresponding ^{13}C chemical shifts for the Z-compound are expected at ca. 23.2 and 32.3 ppm, respectively (9). This compound is thus β-springene(**1**) which also has been isolated by Burger et al.(9) from mammalian secretions of springbok. These workers also identified the two α-isomers by comparing the retention times of products they synthesized from farnesyl acetone (10). However, they did not manage to isolate these isomers in a pure state due to their inherent instability. A triene also was detected.

GCMS revealed the presence of at least four isomeric $C_{20}H_{32}$ compounds, and the mass spectra of these isomers were very similar, with typical fragmentation patterns of isoprenoid hydrocarbons. Thus, fragmentation patterns containing M-69, M-43 and M-15 ion series are observed in addition to the molecular ions. However, based on GCMS alone, all four isomers are not distinguishable because of the facile rearrangement of double bonds in acyclic chains. Based on the relative intensities and NMR spectra, β-springene(**1**), the major component, can be assigned. The other isomers tentatively can be assigned as 2-4 based on their mass spectra and relative retention times as compared to the reported values (10). Additional information was obtained from the ultraviolet spectra of the isomeric mixture of $C_{20}H_{32}$ diterpene hydrocarbons whereby a strong mono- and disubstituted diene absorption at 228.5 nm was observed together with a triene absorption at 256.5 nm. All these spectroscopic and chromatographic data suggested four acyclic diterpene hydrocarbons with the structures 1-4 as shown in Scheme 2.

The presence of these compounds in the PFAD but undetected in CPO and the steam distillate of CPO (8) suggested that they must be degradation products of some natural components. In the unsaponifiable fraction of heat-bleached palm

TABLE II

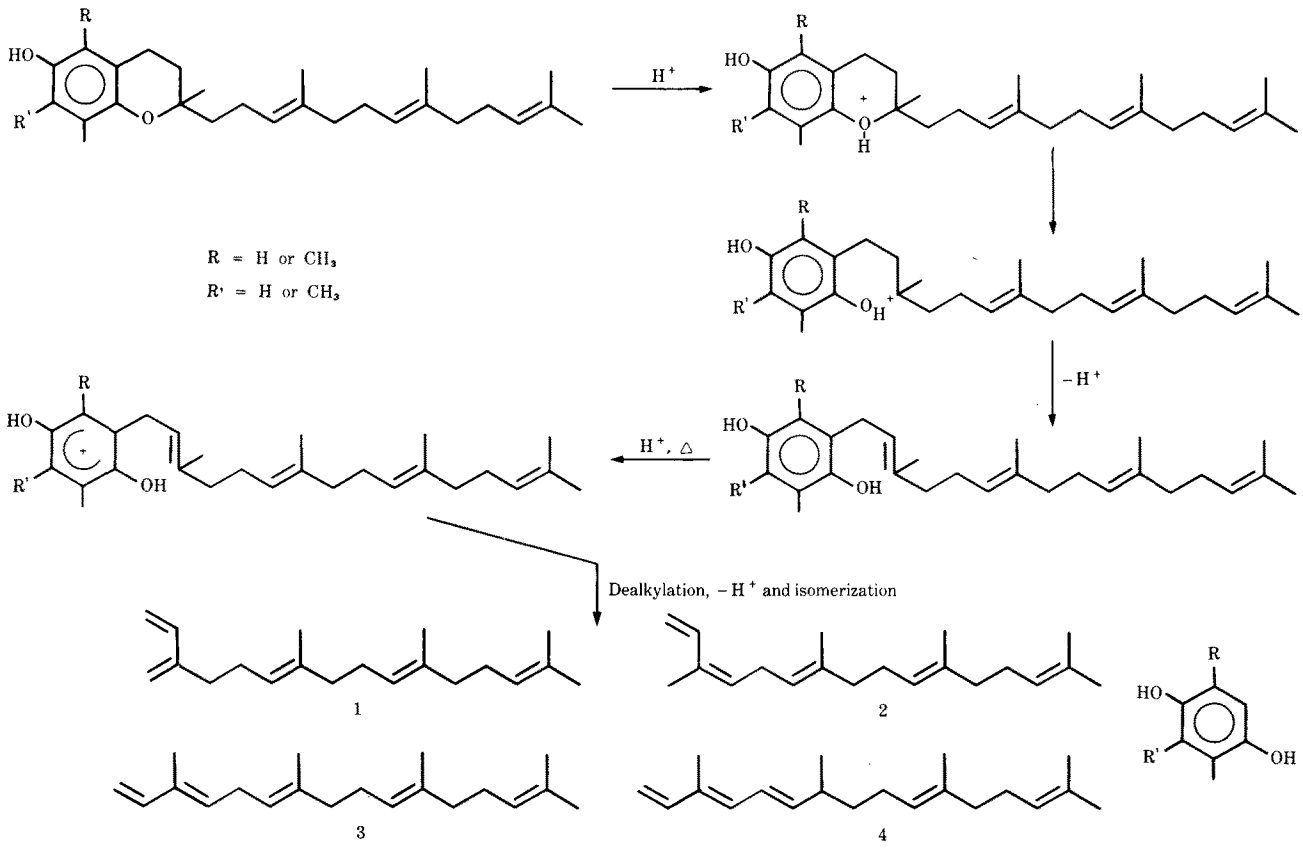
^{13}C Chemical Shifts of (E,E)-7,11,15-trimethyl-3-methylenehexadeca-1,6,10,14-tetraene (1)

Carbon	Observed chemical shift[a] (ppm)	Reported[b] (ppm)
1	115.69	115.63
2	139.03	139.07
3	[c]	146.21
4	31.47	31.52
5	26.69	26.69
6	124.07	124.09
7	[c]	134.93
8	39.75	39.75
9	26.69	26.69
10	124.27	124.29
11	[c]	135.41
12	39.75	39.75
13	26.79	26.82
14	124.41	124.48
15	[c]	131.21
16	25.72	25.66
17	17.68	17.67
18	16.07	16.03
19	16.07	16.03
20	113.05	113.05

[a]$CDCl_3$ solution.

[b]Ref. 9.

[c]These signals for quarternary carbons were too weak for unambiguous assignments.



SCHEME 2. Formation of diterpene hydrocarbons from tocotrienols

oil, Davis et al. (7) detected two $C_{20}H_{32}$ compounds; it seems appropriate to assume that they are the same compounds as two of the isomers that are discussed presently. The formation of these compounds hence is implicated to be derived from thermal "bleaching" or degradation of some natural precursor(s). A search of the natural constituents of palm oil revealed that only three classes of compounds contain isoprenoid units, viz. carotenes, squalene and tocotrienols. Thermal bleaching of carotenes is known (5) to give a series of products such as toluene, xylenes, dimethylnapthalene and ionene, which also are detected in the present work. However, the specificity of degradation of carotenes and squalene to acyclic diterpenes seems unlikely and has not been reported in the literature. Thermal pyrolysis of tocopherols was reported to yield 2,6,10,14-tetramethylpentadecene-1 (11), whereas the pyrolysis of tocotrienols has not been described. A plausible mechanism for the formation of acyclic diterpenes from tocotrienols to yield several isomeric diterpene hydrocarbons is proposed in Scheme 2. In CPO, tocotrienols constituted about 70% of the total tocopherol content (800-900 ppm). Assuming that all the tocopherols and tocotrienols are distilled over together with the free fatty acids (ca. 5% of CPO) and all the tocotrienols are decomposed into diterpene hydrocarbons, the total amounts of these hydrocarbons can be estimated to be 0.74-0.83% of the PFAD. However, it is known that not all the tocotrienols are distilled or decomposed during refining. Therefore, the amounts of these diterpene hydrocarbons detected could be useful in the estimation of the amounts of tocotrienols being decomposed during refining. It may be added that tocotrienols are valuable products to be preserved in refined palm oil in order to achieve a more stable (as they are the principal antioxidants) and more nutritious (as Vitamin E) oil. Also, if they are not decomposed but remain in substantial amounts in PFAD, a useful source of Vitamin E will become available.

The other possibility is that the diterpene hydrocarbons do not occur in free form in the crude palm oil but as ester, alcohol or other derivatives which can suffer ready elimination on heating and distillation. This remains a possibility due to the fact that the more saturated diterpene dienes ($C_{20}H_{38}$) predicted by the mechanism of Scheme 2 to be formed from α-tocopherol, the second dominant component of tocopherols and tocotrienols, were not observed. However, it was observed that the α-isomers of tocopherols and tocotrienols are relatively more stable compounds compared to other isomers.

n-Alkanes, isolated from crude palm oil, are likely to be natural products. They form a complex mixture with masses of 170 to 506. GCMS of n-alkanes showed fragments that are almost identical at low mass fragments, having m/z 57 as the base ion. Prominent ions are of the C_nH_{2n+1} series (71, 85, 99, 113, 127, 141, etc.), and less intense ions are of the C_nH_{2n-1} series (55, 69, 83, 97, 111, 125, 139, etc.). Clusters of smaller peaks were observed around these two ion series and were always more prominent at lower m/z values. The fragment intensities of the C_nH_{2n+1} peaks also decrease in a smooth curve down to M-C_2H_5, and molecular ions are usually distinct but occasionally not observed in long chain n-alkanes (above $C_{25}H_{52}$), especially when the amounts are very small. Branched alkanes were not obtained because if there are any branched methyl or other alkyl groups along the hydrocarbon chains, a completely different fragmentation pattern of ions may be expected (12). Other than the fact that molecular ions are sometimes not observed and the relatively more intense M-15 peaks, the rest of the spectral characteristics are iden-

tical to the reported spectra for n-alkanes (12). It may be noted that nonacosane, hentriacontane and triacontane were not detected in amounts expected from plant sources where palmitic and stearic acids predominate. This would indicate that the hydrocarbon biosynthesis in palm fruits is unrelated to that for fatty acids.

The mass spectra of alkenes from palm fatty acid distillate are similar to those of n-alkanes except that the most abundant ion series is the C_nH_{2n-1} series (55, 69, 83, 97, 111, 125, 139, etc.), m/z 55 is always the base ion and molecular ions are always observed. The smooth curve of decreasing fragment intensities again indicated that there is no branching along the hydrocarbon chains. The position of double bond in the alkenes cannot be located by the mass spectra because of its facile migration in the mass spectrometer. However, proton NMR of the mixture revealed a triplet at 5.35 ppm (J 4.5 Hz), indicating that the double bond in the alkene is of a nonterminal type; no further attempt was made to locate the exact position or positions of the double bond in all the alkenes.

Small amounts of alkadienes (mainly $C_{23}H_{44}$ and $C_{26}H_{50}$) were obtained. The mass spectra of alkadienes are very similar to alkynes having m/z 67, 81, 95, 109, etc. as prominent peaks which are two masses lower than the corresponding alkenes or four masses lower than the alkanes. The assignment of alkadienes rather than the alkynes is made because we observed olefinic protons but no acetylenic or methylene protons allylic to the triple bonds in the proton NMR spectrum of the fraction containing di- and trienes. No further studies (such as microozonolysis and oxidation) were carried out on these compounds. The mass spectra of the aromatic hydrocarbons (xylene, dimethylnaphthalene and ionene) were found to be identical to the reported spectra (12). Apart from squalene, the majority of the hydrocarbons (alkenes and diterpenes) in palm fatty acid distillate as discussed above contain degradation products from tocotrienols, carotenes, fatty acids and perhaps many other components.

ACKNOWLEDGMENTS

The University of Malaya and the Palm Oil Research Institute of Malaysia provided support.

REFERENCES

1. Fitelson, J., *J. Assoc. Off. Agric. Chem. 26*:506 (1943).
2. Dickhart, W., *Am. J. Pharm. 127*:359 (1955).
3. Malaysian Oil Palm Growers' Council, *Malaysian Palm Oil, Technical Bulletin No. 1,* Kuala Lumpur, 1973.
4. Argoud, S., *Oleagineux 9*:789 (1954).
5. Becker, E., I. Mader, H.E. Rost and P. Vogel, *Z. ErnahrWiss. 7*:98 (1966).
6. Rost, H.E., *Chem. Ind. 612* (1976).
7. Davis, J.B., J.M. Robinson, N.K. Silva and A. Barranco, *J. Food Technol. 14*:153 (1979).
8. Dirinck, P., L. Schreyen, L. De Schoemacker, F. Wychuyse and N. Schamp, *J. Food Sci. 42*:645 (1977).
9. Burger, B.V., M. le Roux, H.S.C. Spies, V. Truter and R.C. Bigalke, *Tetrahedron Lett. 52*:5221 (1978).
10. Burger, B.V., M le Roux, H.S.C. Spies, V. Truter and R.C. Bigalke, *Z. Naturforsch. 36c*:340 (1981).
11. Mayer, H., P. Schudel, R. Ruegg and O. Isler, *Helv. Chim. Acta 46*:963 (1963).
12. Stenhagen, E., S. Abrahamsson and F.W. McLafferty, *Registry of Mass Spectral Data,* John Wiley & Sons, New York, 1974.

[Received July 30, 1985]

Effect of Food Emulsifiers on Polymorphic Transitions of Cocoa Butter

Nissim Garti, Judith Schlichter and **Sara Sarig**
Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

The polymorphic behavior of cocoa butter in the presence of several food emulsifiers serving as crystal structure modifiers was investigated. Emphasis was placed on transitions among the relatively stable forms IV, V and VI, which are significant for a confectionery industry.

As known from industry work, within the series of sorbitan esters and ethoxylated sorbitan esters, the solid emulsifiers were the most efficient in retarding transition of V form into VI modification. Blends of sorbitan monostearate (Span 60), ethoxylated sorbitan monostearate (Tween 60) and Span 60-Tween 65 used in the present study were particularly effective. Surprisingly, it was found that some combinations of emulsifiers accelerate the transition of form IV into form V. Transition of form V into form VI occurs via the solid state, and other transitions are known to take place via the liquid phase. Emulsifier was found to increase liquid fraction of the fat prior to its transition. Mechanistic considerations concerning these transitions are suggested.

As known from industry work, chocolate bloom is caused by separation of cocoa butter, the main fatty constituent, from the brown nonfat phase. This phenomenon, occurring more frequently in summer, is deleterious for many chocolate products and seems to be due to the complex polymorphic character of that fat. The phenomenon of polymorphism is known in triglycerides, and it has been studied from the crystallographic point of view (1-3) and with respect to its thermal behavior (4-8). Cocoa butter is a typical example of certain vegetable fats high in 2-oleo-disaturated triglycerides that exhibit complex polymorphism and conditions for isolating each polymorph have been described by other investigators (9,10).

Six polymorphs of cocoa butter are known, distinguishable by melting point, which rises with thermodynamic stability (9,10). Considering that during storage of chocolate, monotropic transition from the V form to the VI form was observed with simultaneous bloom occurrence (11), our study focused on the crystalline forms significant to the confectiner, i.e., forms IV, V and VI and the effect of emulsifiers on their stability and transformation rates. A blend of Span 60 and Tween 60 revealed itself to be a very good bloom inhibitor when added to chocolate (12), but the manner of fat protection is not completely understood.

In earlier publications, it was demonstrated that emulsifiers may be used as crystal structure modifiers in stearic acid; their presence retarded transformation from the unstable to the stable form in stearic acid crystallized from solvents (13,14). In another study we expanded the investigation of the effects of