Journal of Chromatography, 236 (1982) 403–419
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,431

CHARACTERIZATION OF COMMERCIAL WAXES BY HIGH-TEMPERATURE GAS CHROMATOGRAPHY

J. F. LAWRENCE*, J. R. IYENGAR, B. D. PAGE and H. B. S. CONACHER
Food Research Division, Food Directorate, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Canada)
(Received September 29th, 1981)

SUMMARY

Sixteen different waxes (including two oils) were characterized by temperature-programmed gas chromatography up to 400°C, using flame ionization detection. Chromatographic patterns were obtained for the untreated samples and for samples treated with diazomethane and acetic anhydride. Included with the patterns are physical characteristics, origins of the waxes, and where available, references to gas–liquid chromatographic work done by others.

INTRODUCTION

Natural waxes, both animal and vegetable, have been used by man for centuries because of their many useful properties. In nature, waxes serve to protect plants against environmental influences such as microorganisms and drying. Waxes are extremely stable substances with high resistance towards both chemical and biological degradation. Because of this, waxes have found extensive use in the protection of food.

Chemically, natural waxes consist mainly of even-numbered straight-chain carboxylic acids and alcohols in the region of *ca.* C_{12}–C_{36}. Hydrocarbons including both odd numbered and unbranched compounds in the same chain-length region are also usually present[1–5]. Although the composition of the fatty acids in waxes is similar to those found in animal fat used for human nutrition, the latter are usually present as glycerides whereas the former are generally esterified with the long-chain alcohols.

The initial analysis and characterization of waxes, particularly natural waxes, were limited to the estimation of acids, esters and a few other constituents. The analysis of hydrolysis products of Japan wax demonstrated that it was a triglyceride wax[6], while sugar cane wax contained a large portion of polyaldehydes[7].

Thin-layer chromatography (TLC) has been used in the past for characterization of waxes and wax constituents[8–10]. Holloway and Challen[11] carried out a systematic TLC study of a number of wax constituents and applied the technique to the characterization of natural waxes from various sources. Their technique can be particularly useful when used complementary with gas chromatography for the identification of waxes of unknown origin.

0021-9673/82/0000–0000/$02.75

Several published reports have shown that gas–liquid chromatography (GLC) employing high temperatures could be useful for the analysis of glycerides, waxes and some polymers[12–14]. However, with the exception of the work of Tulloch[13] and Valmalle and Karleskind[14] no extensive characterization of commercial waxes has been reported. Tulloch[12,13] reported GLC patterns only after diazomethane and acetylation, while Valmalle and Karleskind[14] carried out direct analysis using a 2% JXR column programmed to 380°C.

The purpose of the present work is to obtain GLC "finger-print" patterns on commercial wax samples to permit the ready identification of the waxes used in industrial and, in particular, food products. Because of the nature of the waxes, special GLC conditions are required, particularly the need for a column stationary phase stable up to 400°C. This report contains a comprehensive account of the GLC patterns of a number of waxes in order that they may be identified in unknown samples. This in-depth characterization in most cases includes patterns obtained by: (a) direct injection of the wax without chemical treatment; (b) after treatment with diazomethane; (c) after treatment with diazomethane and acetic anhydride. The direct analysis yields the hydrocarbon and mono-ester patterns while the diazomethane treatment will include the free fatty acids (as methyl esters). Finally, the free alcohols present will appear as acetates after treatment with acetic anhydride. The resulting three chromatograms yield valuable information useful for characterizing the many different commercial waxes.

EXPERIMENTAL

Apparatus

Gas chromatography was carried out using a Varian Model 2700 gas chromatograph with flame ionization detector. Columns were 3 ft. × 1/8 in. I.D. stainless steel, packed with 80–100 mesh, acid-washed and silanized Chromosorb W coated with 1.5% Dexil 300. The temperature was programmed from 150 to 400°C at 4°C/min. Helium carrier gas flow-rate was 60 ml/min. Detector attenuation was 32×10^{-11}. Injection volumes were normally 3–5 μl of a 1% wax (or oil) solution.

Esterification

Methyl esters of the fatty acids were formed according to the method of Tulloch[9], briefly described as follows. Wax (50 mg) was weighed and dissolved in 5 ml of chloroform. Freshly made diazomethane in ether was then added and the mixture refrigerated overnight. The solution was evaporated to dryness, then dissolved in 5 ml of chloroform for chromatographic analysis.

Acetylation

The solution from above was evaporated to dryness and 1.0 ml of acetic anhydride followed by 1.0 ml of anhydrous pyridine ("Pyridine-Plus"; Pierce, Rockford, IL, U.S.A.) were added to the residue. The contents were mixed and left at room temperature overnight. The solvent was removed by evaporation and the residue dissolved in chloroform for analysis.

RESULTS AND DISCUSSION

Paraffin waxes

Paraffin waxes are prepared under pressure from paraffinic hydrocarbons[15]. They are mixtures of solid saturated aliphatic hydrocarbons with melting points in the range 43–65°C. Their molecular weights vary from 300 to 500 with chain lengths of 20 to 35 carbon atoms. Figs. 1 and 2 shows the patterns obtained from two paraffin waxes (m.p. 53°C, with carbon chain maximum around C_{23}–C_{25}; m.p. 58°C, carbon chain maximum near C_{26}–C_{28}). The figures show that the two waxes have the same hydrocarbon composition but with varying maxima, the lower maxima being found for the lower-melting waxes. A GLC tracing of a 53°C paraffin wax obtained earlier[14] is similar to the one shown in Fig. 1.

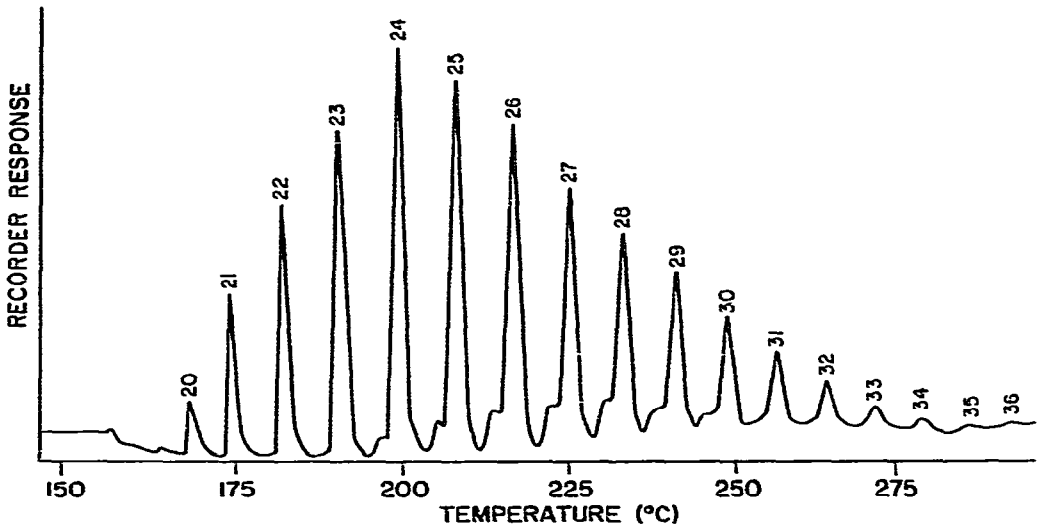

Fig. 1. Paraffin wax, melting point 53°C.

Microcrystalline waxes

After distillation of petroleum waxes, the residues obtained after crystallization are microcrystalline waxes which are solid hydrocarbons. They are made up of saturated hydrocarbons containing 25–50 carbon atoms with a melting point greater than 65°C. Their molecular weight exceeds that of the paraffins (400–700).

Figs. 3 and 4 show the curves obtained for the two microcrystalline waxes (types 1140/10 and 1251/7), showing the sequence of saturated hydrocarbons with a maximum around C_{38}–C_{42} and C_{32}–C_{35} for waxes 1140/10 and 1251/7, respectively. Valmalle and Karleskind[14] reported a similar GLC pattern of this type of wax.

German montan waxes

These are considered fossil vegetable waxes[16] (also considered mineral waxes) obtained by extraction of coal or lignite. The wax is purified by vacuum distillation or by solvent processing.

It has been reported by Lange and Wildgruber[17] that the main components of

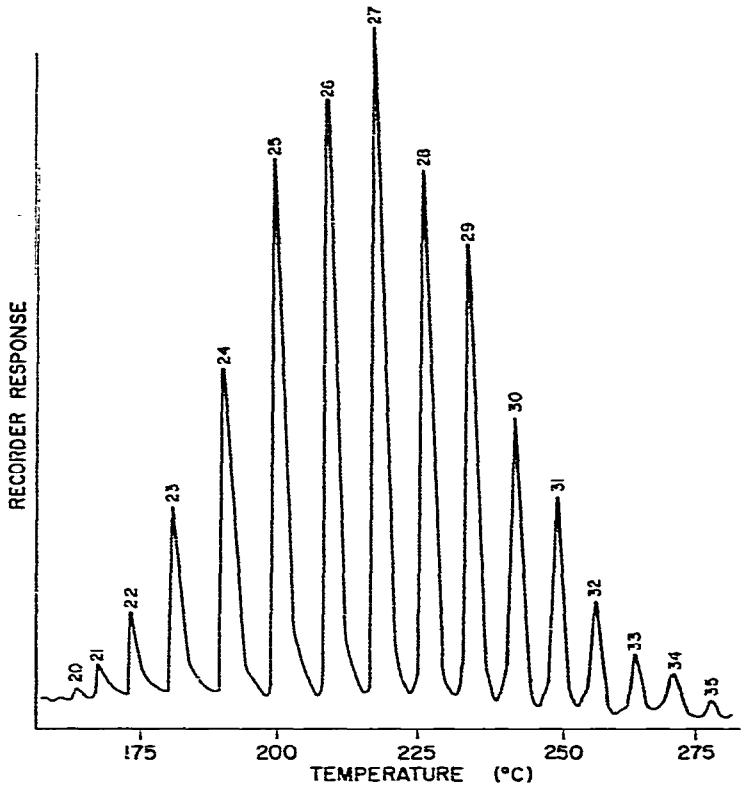

Fig. 2. Paraffin wax, melting point 58°C.

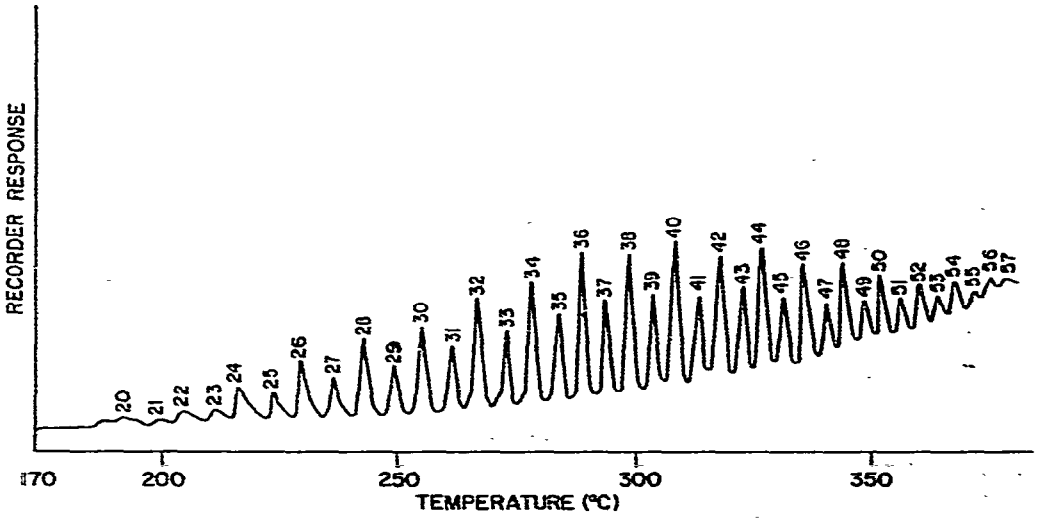

Fig. 3. Microcrystalline wax, type 1140/10.

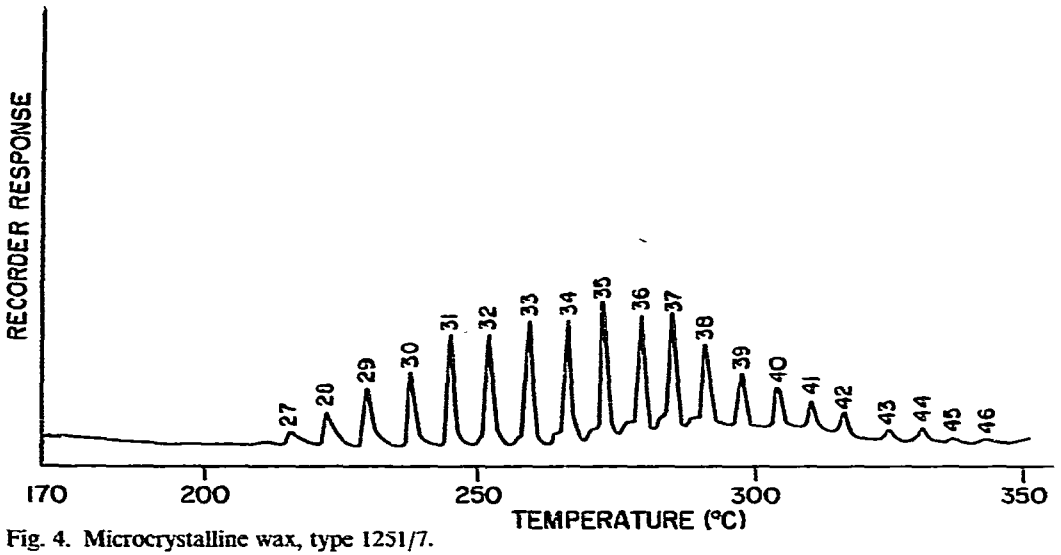

Fig. 4. Microcrystalline wax, type 1251/7.

montan waxes are esters of the C_{28} and C_{30} acids as well as C_{28} and C_{30} alcohols. Valmalle and Karleskind[14] reported the following composition for a sample of the wax: esters, 50%; alcohols, 1–2%; resin and asphalt, 25%; unidentified, 8%.

Fig. 5 shows chromatographic results before and after the treatments outlined in the Experimental section. The untreated wax shows mainly hydrocarbons in the region C_{25}–C_{33}. The peaks in the region C_{50}–C_{62} represent monoesters which remain unaffected after the chemical treatments. The diazomethane and the diazomethane–acetylation treatments show the fatty acids (methyl esters) and alcohols (acetates) contained in the sample. The major acid peaks shown in Fig. 5 are C_{26}, C_{28} and C_{30} acids. Montanic acid is the C_{28} peak, usually the most predominant constituent[17].

Beeswax

This is a digestion product secreted by the common *Apis mellifera* bee. A yellow wax, it can be bleached by various decolorization processes (solar UV radiation, conventional decolorizing earth, active charcoal) without significantly changing its composition.

It has been reported by Tulloch[13] that only 63% of the components in beeswax are volatile after diazomethane and acetylation treatment and it consists of 15% hydrocarbons, 12% acids and 36% long-chain esters.

Fig. 6 shows GLC patterns obtained with yellow beeswax, while Fig. 7 shows the corresponding results for white (decolorized) beeswax. For the latter, the acetylation treatment produces no significant change from the diazomethane results indicating the absence of alcohols in the C_{20}–C_{35} region. It has been reported that the chromatographic patterns of beeswax (white) are rather consistent regardless of geographical origin[14,18]. The yellow beeswax differs from white beeswax in the short-chain fatty acid composition as well as in the presence of some alcohol peaks.

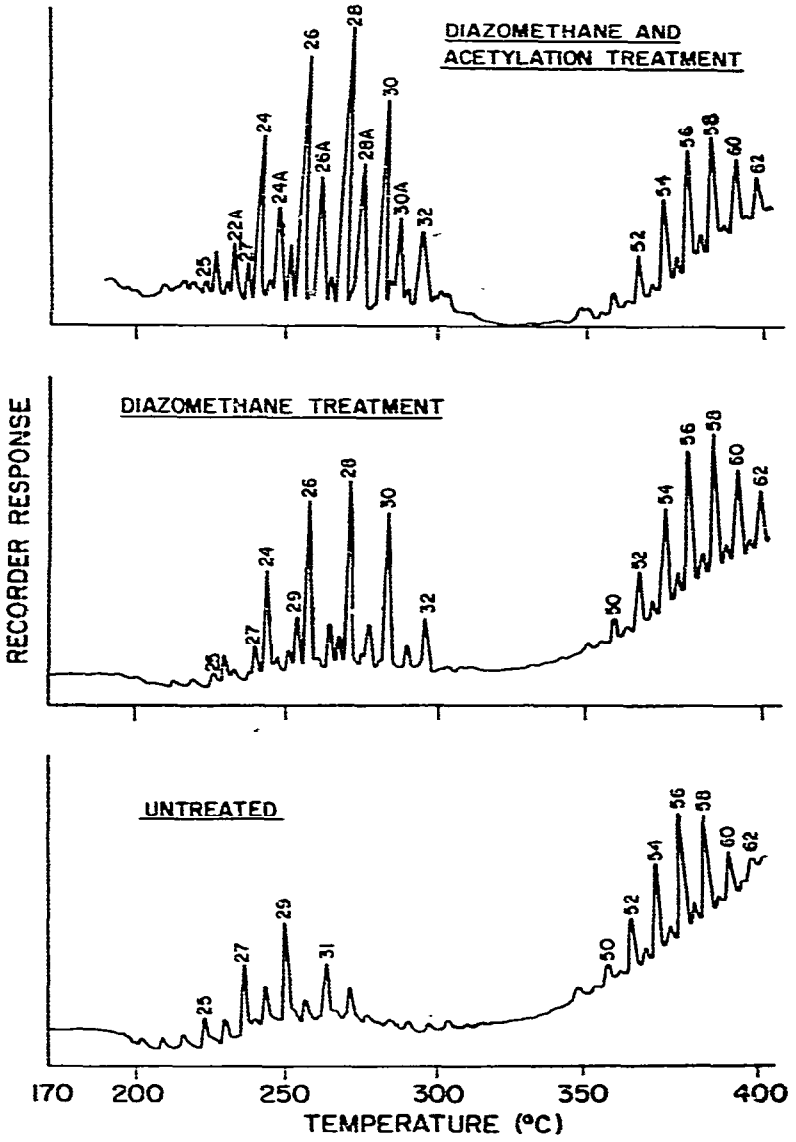

Fig. 5. German montan wax.

Spermacetti substitute

Spermacetti is a whitish hard wax obtained from the head of the sperm whale. It consists of over 95% esters and some alkanes, fatty acids, and *ca.* 3% alcohols. Of the esters, chiefly cetyl palmitate (C_{32}) is present in appreciable amounts. Esters of lauric, stearic and myristic acids and esters of higher alcohols are also present.

The substitute received by us is an artificial mixture prepared and sold in place of spermacetti due to its increasing cost and limited availability. From Fig. 8 it can be seen that the spermacetti substitute when injected without any treatment shows a

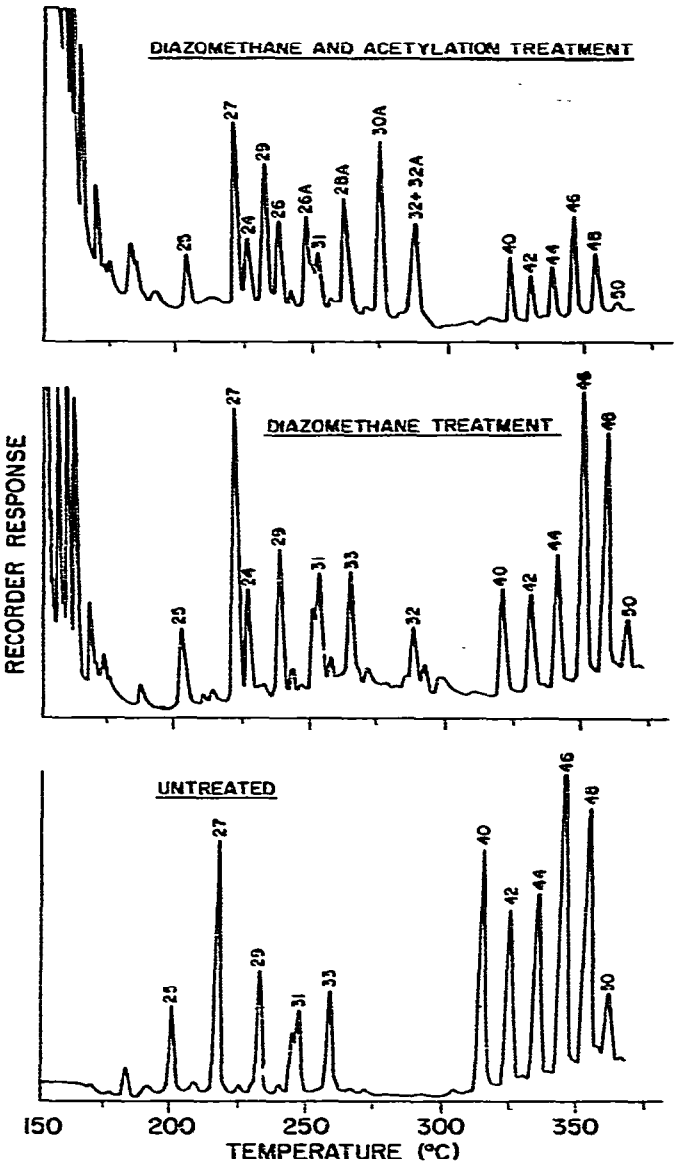

Fig. 6. Yellow beeswax.

maximum at C_{32}, similar to that found by Valmalle and Karleskind[14] and Holloway[19] for the original wax. After treating the substitute with diazomethane, methyl esters of C_{20}, C_{22}, and C_{24} were observed. After diazomethane and acetyation treatments, some alcohol acetate peaks were observed.

Japan wax

This wax is extracted from the protective coating of Sumac kernels[15].

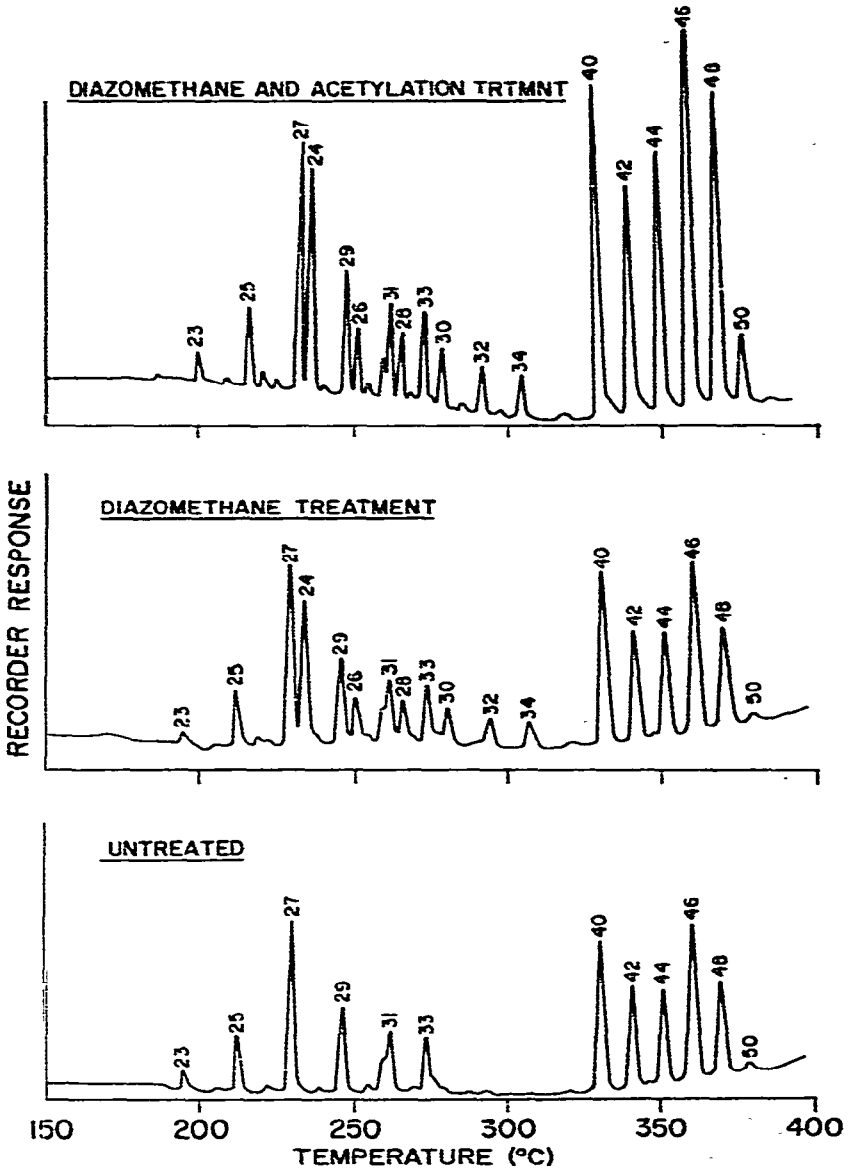

Fig. 7. Decolorized (white) beeswax.

Tsujimoto[6] reported that it is a glyceride wax and gives, on hydrolysis, mainly palmitic acid and 6% long-chain dicarboxylic acids, and on methanolysis gives methyl esters, together with esters of eicosanedioic (0.2%) and docosanedioic (2.6%) acids. Similar percentages of dicarboxylic acids have been reported by Tazaki[20].

Tulloch[13] reported that the volatile amount of Japan wax was 46% and it was composed of: free acids, 3%; triglycerides, 36%; unidentified, 5%. The same author reported that by GLC (diazomethane and acetylation), three glyceride peaks were

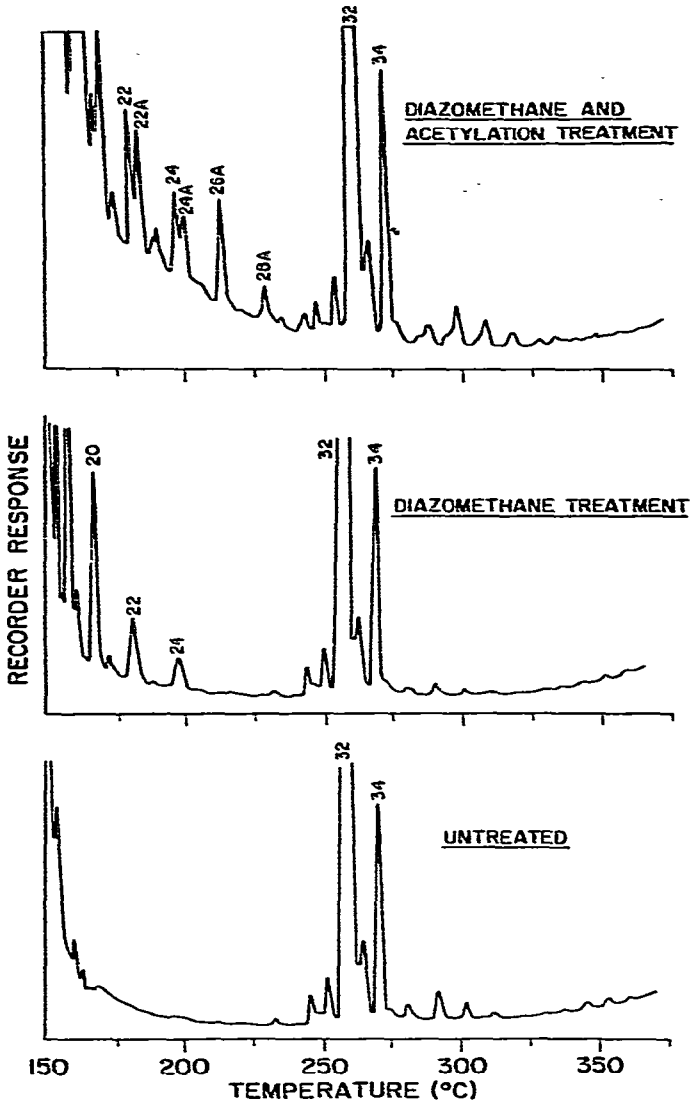

Fig. 8. Spermacetti substitute.

obtained, namely 48G, 50G and 52G, where 48G (tripalmitin) was the major component. Valmalle and Karleskind[14] reported the same composition by injecting the wax without any treatment.

Fig. 9 shows the three chromatograms for Japan wax. The untreated chromatogram is somewhat different than that obtained by Valmalle and Karleskind[14] and Tulloch[13] in that they report 48G (glyceride) as the predominant peak and few constituents below this in carbon-chain length. Fig. 9, untreated, indicates the presence of traces of hydrocarbons in addition to the glyceride peaks. The differences here might be explained by the age of the kernels before extraction of the wax. Tulloch[21] showed

through his work on leaf wax of Selkirk variety spring wheat, that from 44 days to 100 days the hydrocarbons, alcohols and monoesters disappeared to a great extent, giving rise to long-chain esters and diesters with 2,3-unsaturations.

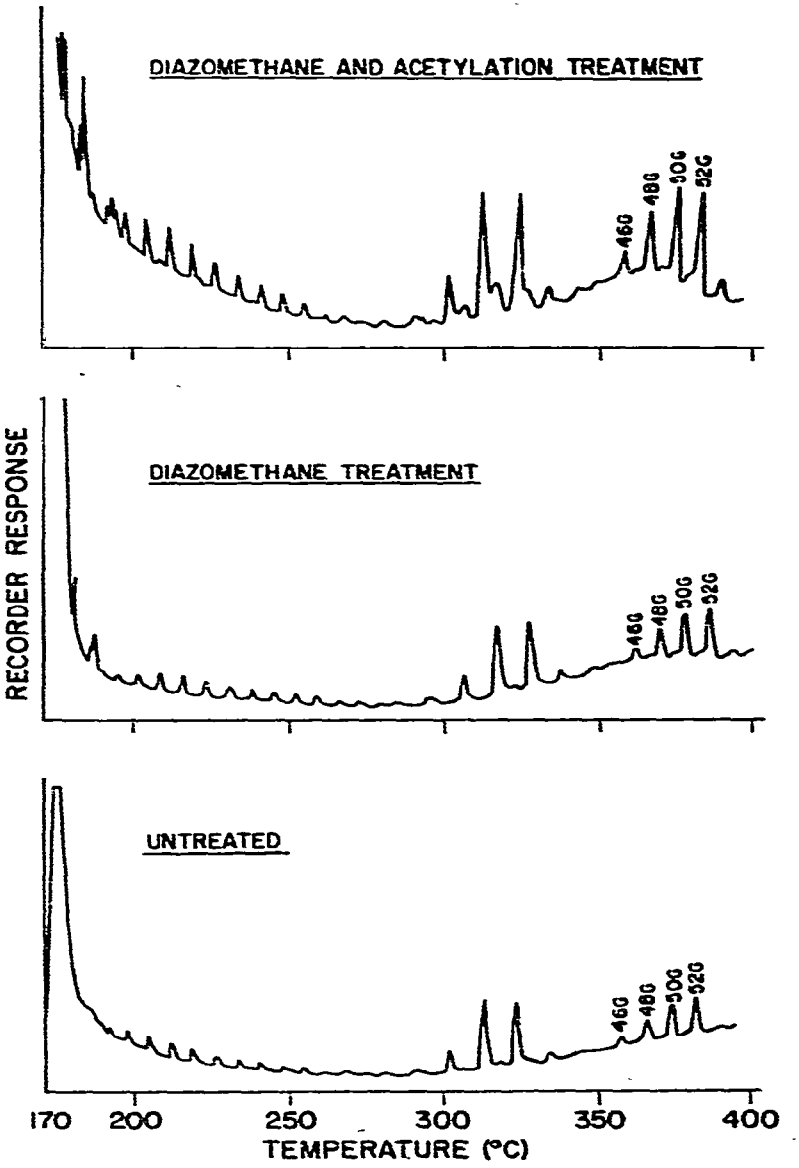

Fig. 9. Japan wax.

Candelilla waxes

These waxes are extracted from various plants, such as *Euphorbia antisiphilitica*, that grow in Southern Texas and Northern Mexico. They are yellow waxes containing *ca.* 40–65% hydrocarbons, 10% aliphatic alcohols, 30% esters and 8–10%

fatty acids[14]. Tulloch[13] reported that 65% of the wax is volatile on GLC after diazomethane and acetylation treatments. The hydrocarbon content of this wax was reported to be *ca.* 41–55% with C_{31} as the principal component, being nearly 80% (refs. 22–24).

Fig. 10 compares the results of the chemical treatments. It can be seen that the components of the wax consists of hydrocarbons, some fatty acids and alcohols all in the region of C_{23}–C_{34}. These results agree with results reported earlier[13,14,21,25].

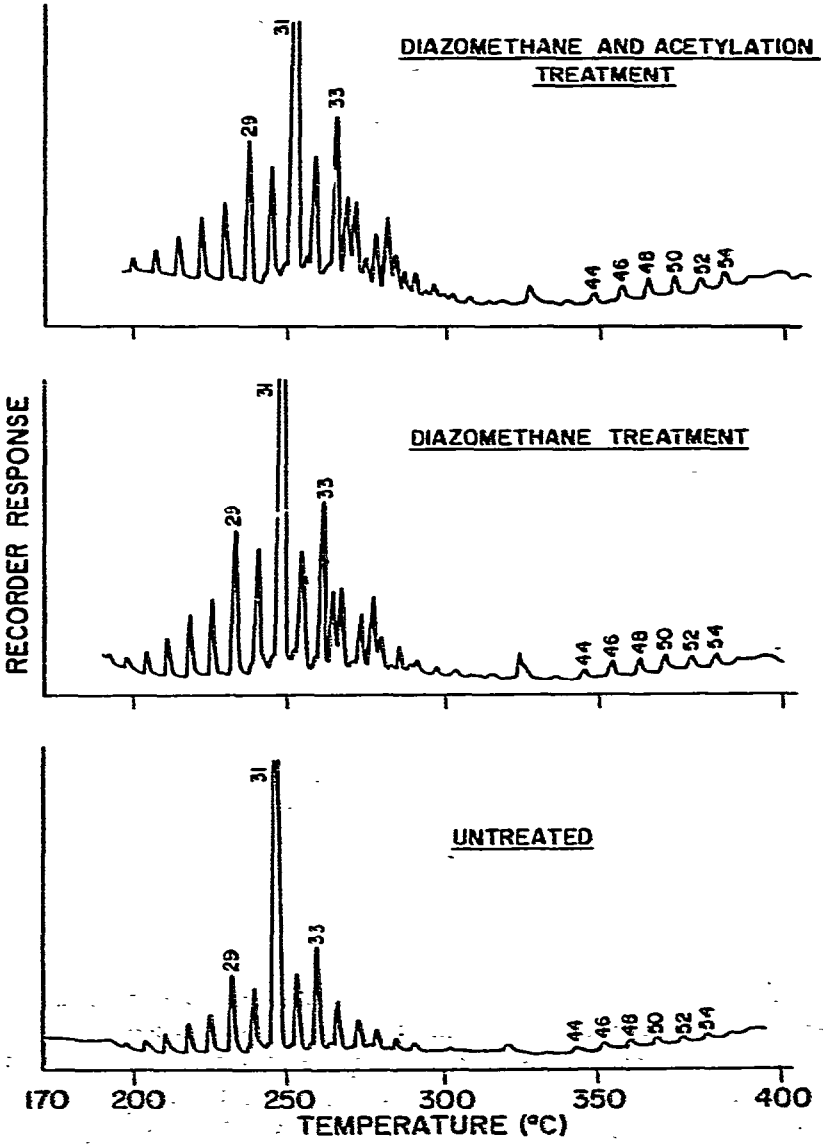

Fig. 10. Candelilla wax.

Ouricury wax

Because of its physical properties, this wax has often been considered a substitute for Carnauba wax. However, the GLC patterns are significantly different. Fig. 11 shows the chromatographic results obtained for ouricury wax.

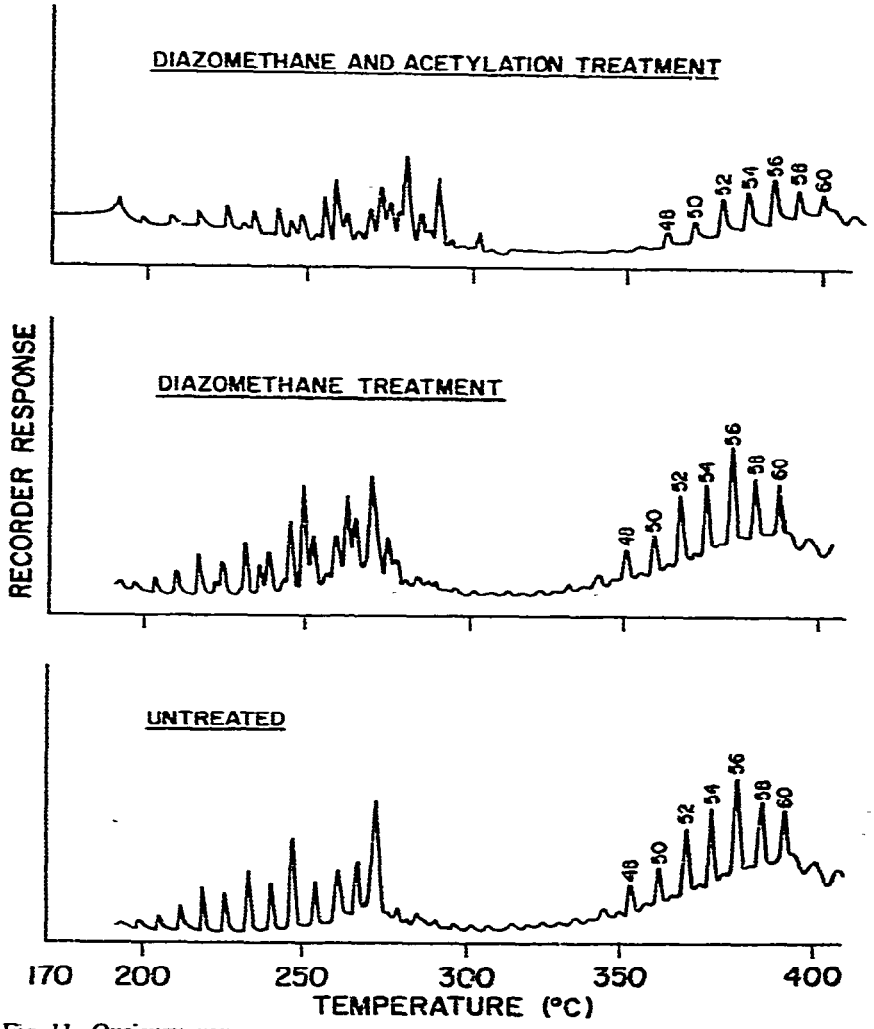

Fig. 11. Ouricury wax.

Carnauba wax

This is one of the most useful and valuable waxes and is extracted from leaves of *Cerifera* palm trees[26]. Carnauba is the highest melting of the commercial vegetable waxes (83–86°C m.p.), a fact which accounts for many of its uses. It is an important component of polishes, and is also used in lipsticks and cosmetic creams, glazing for paper, pills and candies, carbon paper, ink, candles, varnish and enamel[26].

It has been reported by Tulloch[13] that only 47% of the components in Car-

nauba wax are volatile after diazomethane and acetylation treatments, which consist of 11% alcohols and 36% esters. According to Valmalle and Karleskind[14], the composition of Carnauba wax is as follows: esters (*ca.* 80%), alcohols (10–15%), free acids (3–5%) and hydrocarbons (2–3%). Fig. 12 shows the chromatograms obtained for this wax. The untreated wax shows hydrocarbons and monoesters C_{48}–C_{64} with a maximum at C_{56}. No change is observed after diazomethane treatment; however, four alcohols (peaks C_{28}, C_{30}, C_{32}, C_{34} with a maximum around C_{32}) are observed after

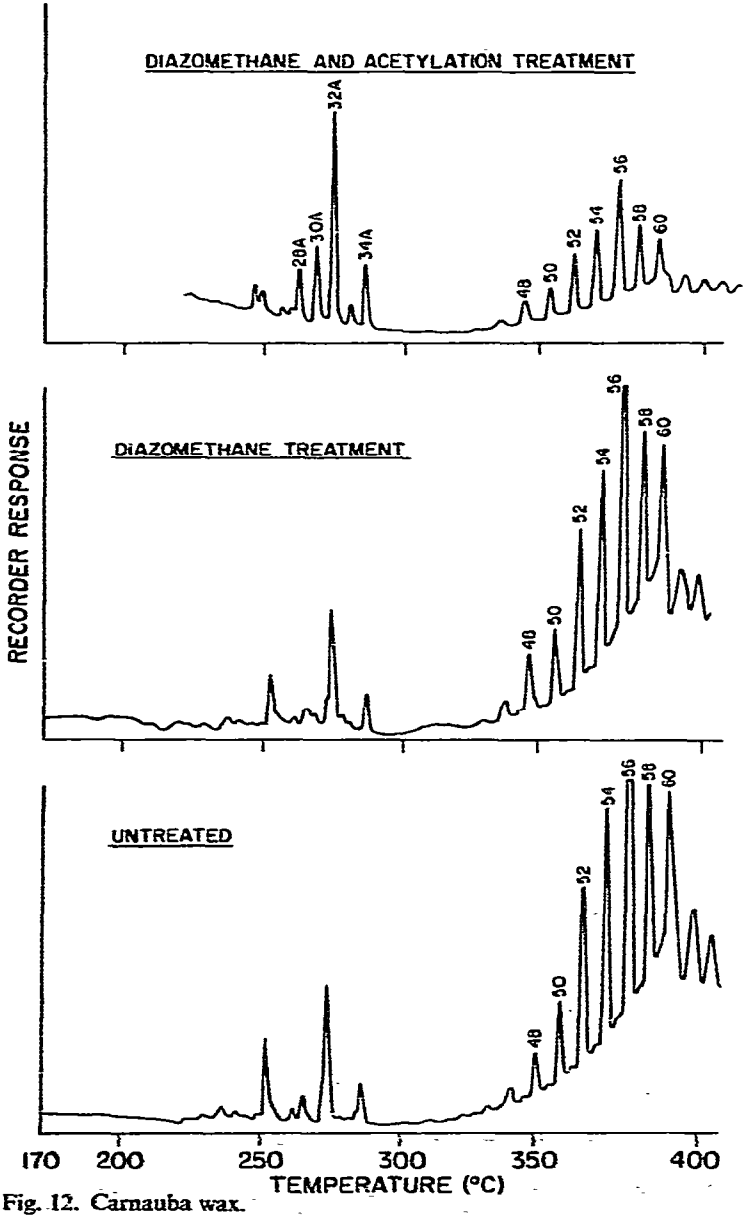

Fig. 12. Carnauba wax.

acetylation (one third or original sample size injected). This is similar to results reported earlier[13,14].

Rice bran wax

Rice bran wax is, as the name suggests, isolated from rice bran oil, which is a commercially important source of edible oil. The purified wax is hard, slightly crystalline and varies in color from tan to light brown. No GLC patterns have been reported up to the present. Fig. 13 shows the results obtained with and without chemical treatment. There is little difference between the untreated and diazomethane extracts. However, acetylation does show at least four alcohols.

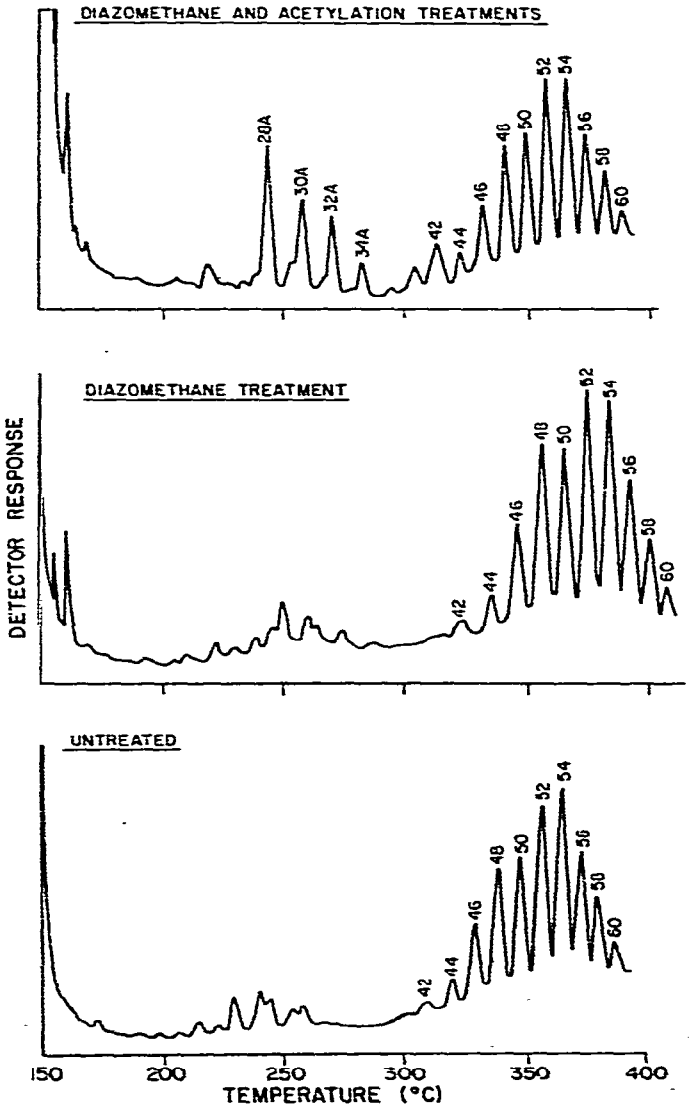

Fig. 13. Rice bran wax.

Shellac wax

Shellac wax is derived from *Tachardia Lacca* and has been studied by several groups by classical means[29–31]. Tulloch[13] has shown that after diazomethane and acetylation the GLC indicated a large free alcohol content. This is also observed in Fig. 14; however, there is little difference between the untreated and diazomethane results.

Mineral and paraffin oils

These oils consist largely of saturated straight-chain (C_{14}–C_{18}) and cyclic hydrocarbons. They are extensively used in baking operations and for fruit and

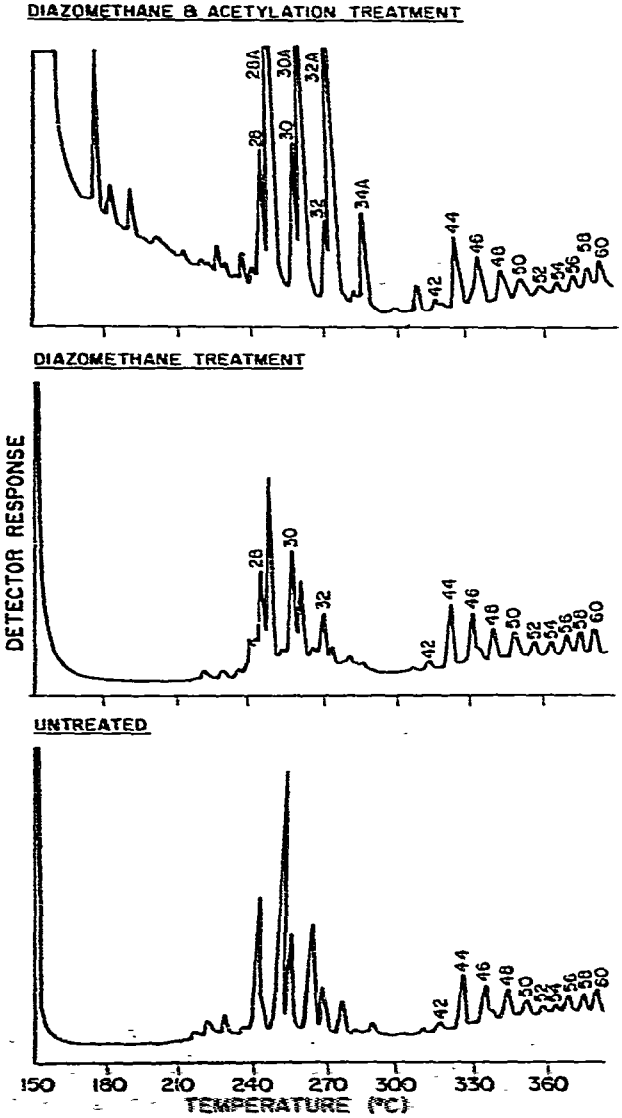

Fig. 14. Shellac wax.

vegetable coatings. Fig. 15 shows GLC chromatograms of the untreated oils analyzed under the same conditions as for the waxes. No discernable peaks were observed for the two. Also, no changes were produced as a result of the chemical treatments.

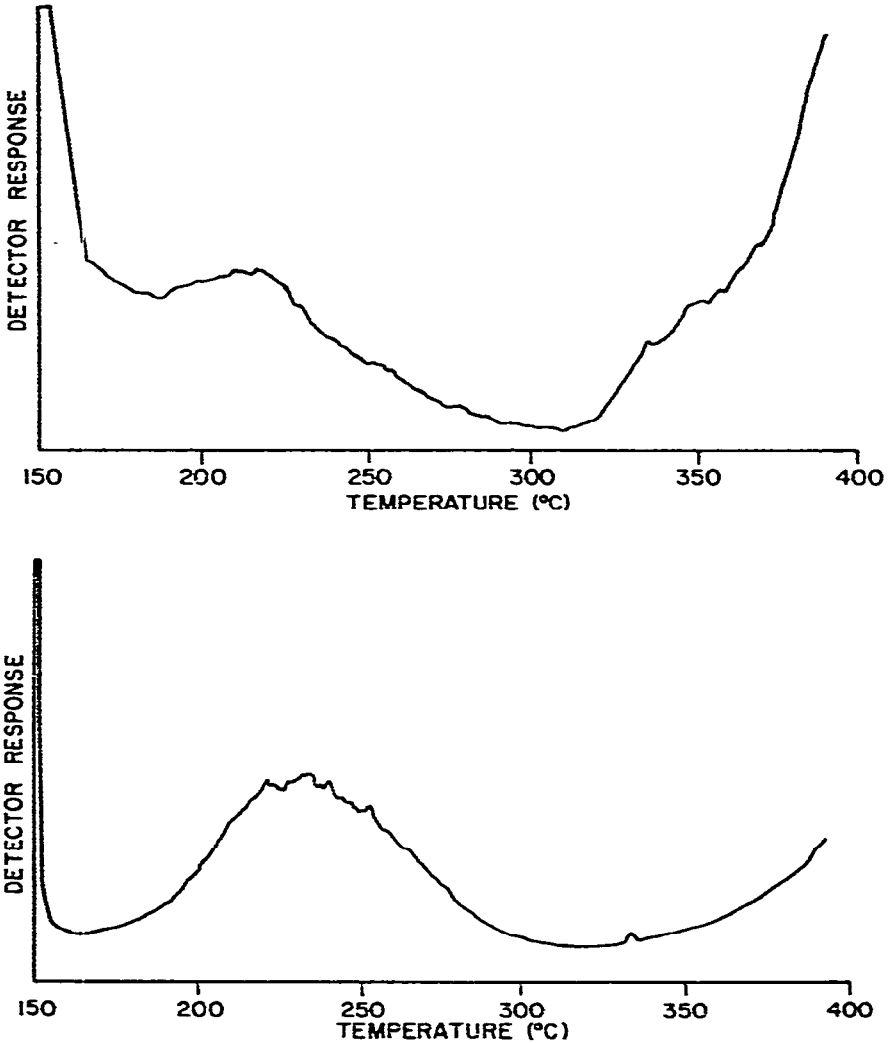

Fig. 15. GLC chromatograms of paraffin oil (top) and mineral oil (bottom).

CONCLUSION

The GLC patterns of fourteen waxes and two oils have been obtained before and after various chemical treatments. These results provide very useful data for the identification of unknown commercial waxes, especially in the food-processing industry where the use of waxes is subject to regulations.

REFERENCES

1 A. H. Warth, *The Chemistry and Technology of Waxes*, Reinhold, New York, 1956.
2 V. Wollrab, *Parfum. Cosmet. Savons*, 9 (1966) 348.
3 F. J. Ludwig, *Soap Chem. Specialties*, 42 (1966) 70.
4 J. B. Alleboone, R. J. Hamilton, B. A. Knights, B. S. Middleditch and D. M. Power, *Chem. Phys. Lipids*, 4 (1970) 37.
5 M. Streibl, K. Stramosky and F. Sorm, *Neuzeitliche Wachsforschung VII*, Vol. 35, Industrieverlag von Hernschaussen KG, Hamburg, 1966.
6 M. Tsujimoto, *Bull. Chem. Soc. Japan*, 10 (1935) 212.
7 J. A. Lamberton and A. H. Radcliffe, *Austral. J. Chem.*, 13 (1960) 261.
8 F. Radler and D. H. S. Horn, *Austral. J. Chem.*, 18 (1965) 1059.
9 H. P. Kaufmann and B. Das, *Fette, Seifen, Anstrichm.*, 65 (1963) 398.
10 W. Hessler and F. Sammet, *Fette, Seifen, Anstrichm.*, 67 (1965) 552.
11 P. J. Holloway and S. B. Challen, *J. Chromatogr.*, 25 (1966) 336.
12 A. P. Tulloch, *J. Amer. Oil Chem. Soc.*, 49 (1972) 609.
13 A. P. Tulloch, *J. Amer. Oil Chem. Soc.*, 50 (1973) 367.
14 G. Valmalle and A. R. Karleskind, *Franc. Corps. Gras.*, 24 (1977) 203.
15 H. Bennett, *Commercial Waxes: A Symposium and Compilation*, Chemical Publishing Co., New York, 2nd ed., 1956.
16 W. Presting and T. Krenter, *Fette, Seifen, Anstrichm.*, 67 (1965) 334.
17 J. Lange and J. Wildgruber, *Fette, Seifen, Anstrichm.*, 78 (1976) 62.
18 A. P. Tulloch, *Cosmetics and Perfumery*, 89 (1974) 53.
19 P. J. Holloway, *J. Pharm. Pharmacol.*, 20 (1968) 775.
20 M. Tazaki, *Kogyo Kagaku Zasshi*, 74 (1971) 524.
21 A. P. Tulloch, *Phytochemistry*, 12 (1973) 2225.
22 P. J. Holloway, *J. Sci. Food Agr.*, 20 (1969) 124.
23 G. Spengler and G. Hauf, *Fette, Seifen, Anstrichm.*, 59 (1957) 607.
24 M. A. Schuette and J. G. Baldinus, *J. Amer. Oil Chem. Soc.*, 26 (1949) 530.
25 A. C. Chibnall, S. H. Piper, A. Pollard, E. F. Williams and P. N. Sahai, *Biochem. J.*, 28 (1934) 2189.
26 United States Tariff Commission, in *Summaries of Trade and Tariff Information, Schedule 4*, Vol. 12, TC Publication 239, Washington, DC, 1968, p. 121.
27 D. T. Downing, Z. H. Kranz and K. E. Murray, *Austral. J. Chem.*, 14 (1961) 619.
28 L. E. Vandenburg and E. A. Wilder, *J. Amer. Oil Chem. Soc.*, 47 (1970) 514.
29 A. Gascard, *Ann N.Y. Acad. Sci.*, 159 (1912) 258.
30 E. Faurot-Bouchet and G. Michel, *J. Amer. Oil Chem. Soc.*, 41 (1964) 418.
31 W. Carruthers, J. W. Cook, N. A. Glen and F. D. Gunstone, *J. Chem. Soc.*, (1956) 5251.