the noble metal catalysts for this reason as well as cost.

These results are at some variance with those of Johnston et al. (15), who found that the selectivity for several commercial platinum catalysts was significantly lower than for palladium catalysts and below seven out of eight nickel catalysts evaluated. However, elevated temp (80–150C) in the present work provided high selectivities for the noble metal catalysts whereas low selectivities were obtained at 35C. In the cited investigation, the noble metal catalysts were evaluated only at 25C. Also the differences may be related to the relative rates of hydrogenation of the methyl esters and of the glyceride ester mixture. Johnston et al. (15) indicated that the hydrogenation of trilinolenin took four to five times as long as the equimolar mixture of methyl linoleate and linolenate required for their procedure (16).

With nickel catalysts, the cited study and portions of the present work were performed at comparable temp (i.e., 140 and 150C, respectively). However, the high selectivities (1.5–2.7) and the high isomerization (18.0–22.8%) were not duplicated. Thus, radical differences in reaction rates of methyl esters and soybean glyceride mixtures may account for the anomalous results recorded. Further study is certainly indicated.

ACKNOWLEDGMENTS

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# Separation of Triglycerides by Column Chromatography on Silica Impregnated with Silver Nitrate[1]

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## Abstract

Silicagel impregnated with silver nitrate was used for the column-chromatographic separation of closely related triglycerides. Good results were obtained with 20–100 mg of the following glyceride mixtures:

1. Dipalmito-elaidin and dipalmito-olein.
2. Tristearin, dipalmito-olein, stearo-diolein and triolein.
3. Dipalmito-olein and dipalmito-linolein.

Six well-separated fractions were obtained by chromatography of palm oil and the total triglyceride composition of the oil was calculated from the composition of the fractions.

## Introduction

AT THE MOMENT the analysis of glyceride mixtures is being thoroughly investigated. Apart from the fatty acid components, which can be easily determined quantitatively by gas chromatography of the corresponding methyl esters, there is an increasing interest in the way in which the fatty acids are combined with glycerol to form glyceride molecules. The availability of more advanced techniques for the determination of the composition of triglyceride mixtures would indeed contribute considerably to our fundamental knowledge of interesting and highly important subjects, such as the metabolism of triglycerides in mammals and the relationship between the triglyceride structure and the consistency and rheology of dietary fats.

In earlier publications (1,2) a new chromatographic adsorbent was described for the separation of higher fatty acid methyl esters according to their degree of unsaturation or according to the configuration (*cis* or *trans*) of their double bonds. The adsorbent, which can also be applied to TLC (3,4,5), owes its high selectivity to the presence of a large amount of silver ions. The separation of mg amounts of closely related triglycerides using this type of adsorbent in column chromatography has been briefly communicated (1). The present report gives a detailed description of the results obtained so far.

## Experimental

*Adsorbent.* The preparation of the chromatographic adsorbent (silica impregnated with silver nitrate) has already been described (2).

*Solvents.* The benzene used was of analytical grade; the dietyl ether distilled before use. The light petroleum was purified from aromates according to the method of Van der Ven et al. (6) and fractionated by distillation. The fraction with a boiling range of 40–60C was used.

*Triglycerides.* The monoacid triglycerides tristearin (SSS) and triolein (OOO) were prepared by means of the reaction between glycerol and acid chloride (7). The asymmetric triglycerides, dipalmito-elaidin (PPE), dipalmito-olein (PPO) and stearo-diolein (SOO), were prepared from the monoglycerides (8) 1-mono-elaidin, 1-mono-olein and 1-monostearin repectively by acylation in the presence of pyridin and

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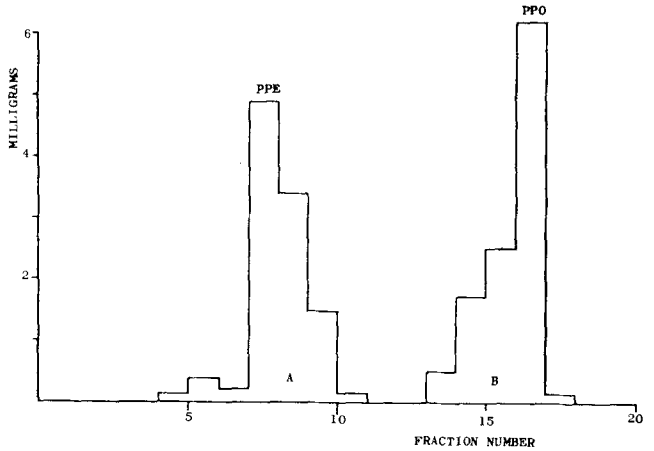


FIG. 1. Chromatography of 10.8 mg each of dipalmito-elaiden (PPE) and dipalmito-olein (PPO). Recovery A, 10.7 mg; B, 10.9 mg. *Trans* double bond content in C 18:1 methyl ester fractions of A and B, 88% and 5%, respectively.

chloroform (9).

A mixture of dipalmito-olein (PPO) and dipalmito-linolein (PPL) was obtained from cottonseed oil. A 100-g portion of the oil was crystallized from 100 ml acetone at −5C. Separated crystals were collected and recrystallized from acetone. In order to remove oxidized triglycerides and mono- and diglycerides, 2 g crystals obtained were purified by column chromatography. A column (in diameter 25 mm) containing 30 g silicic acid (Mallinckrodt, 100 mesh, analytical grade) and 15 g Celite 535 was used according to the method of Quinlin et al. (10). A mixture of diethyl ether and light petroleum (5:95, v/v) was used as eluant. The fraction eluting between 150 and 250 ml was collected and contained 1.8 g oil. The fatty acid composition was (mole %):

| | | | |
|---|---|---|---|
| Palmitic acid | 64.0% | Oleic acid | 10.8% |
| Stearic acid | 2.8% | Linoleic acid | 22.3% |

This analytical result is in good agreement with a PPO/PPL ratio of 1:2. It is supposed that the oleoyl- and linoleyl-groups mainly occupy the β-positions in the triglyceride molecules (11).

*Palm Oil.* An amount of palm oil (2 g neutralized and bleached) was purified by chromatography over silicic acid (see above). The fatty acid composition was (mole %):

| | | | |
|---|---|---|---|
| Myristic acid | 1.0% | Oleic acid | 38.6% |
| Palmitic acid | 44.4% | Linoleic acid | 9.8% |
| Stearic acid | 6.2% | | |

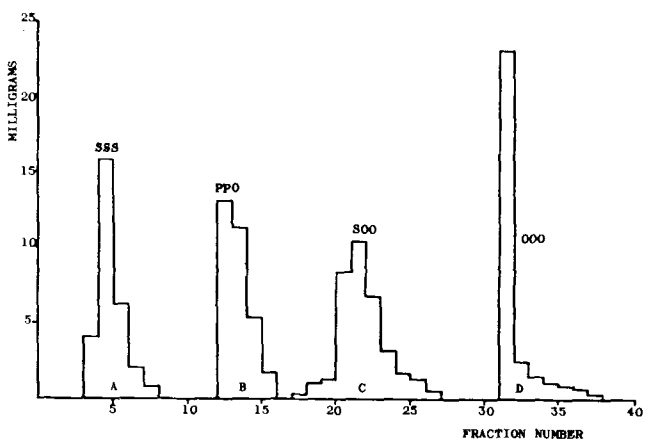


FIG. 2. Chromatography of a mixture of tristearin (SSS) dipalmito-olein (PPO), stearo-diolein (SOO) and triolein (OOO).

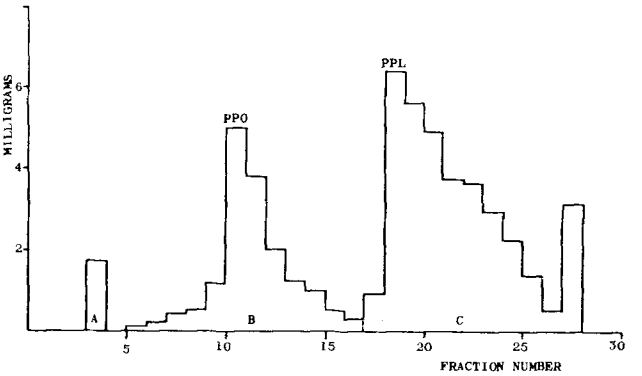


FIG. 3. Chromatography of 50 mg of a mixture of dipalmito-olein (PPO) and dipalmito-linolein (PPL). Fraction A is probably fully saturated triglycerides.

*Column.* A chromatographic column (in diameter 11 mm, effective length 40 cm) provided with a cooling mantle and a Teflon-cock with a capillary attachment was used. A 250-ml reservoir for the eluant was placed on the column by a ground joint. The temp of the column was kept at 15C.

*Elution.* Twenty to 100 mg triglyceride mixture was dissolved in 10 ml light petroleum or, if a considerable amount of saturated triglyceride was present, in a mixture of benzene and light petroleum (20:80 v/v) and subsequently applied to the column. The eluting solvents consisted of mixtures of benzene or diethyl ether and light petroleum. At the top of the column a pressure of 10–15 cm water was maintained while the flow-rate was adjusted at 30 ml/hr. During the chromatographic experiments, 10-ml fractions were collected and separately evaporated to dryness (50C) in weighed small glass dishes by a stream of nitrogen. Subsequently, the glycerides were determined gravimetrically with an accuracy of ca. 0.2 mg. Fractions apparently belonging to one and the same chromatographic peak were combined and coded A, B etc. These fractions were converted into methyl esters and the fatty acid composition determined by means of GLC.

The gas-chromatographic analyses were carried out by a Pye instrument with gas density balance or with an Argon ionization detection. The column (length 120 cm) was packed with polyethylene glycol adipate on Celite (20% w/w). The determination

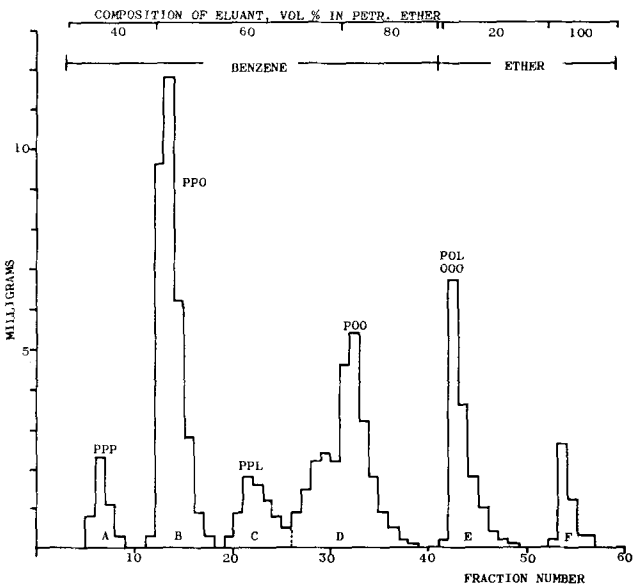


FIG. 4. Chromatography of palm oil (97 mg).

TABLE I

Operational Data on the Chromatography of Triglyceride Mixtures

| Run-number | Triglycerides mg | $AgNO_3$-silica [a] g | Sequence of eluent solvents in light petroleum 40–60C |
|---|---|---|---|
| 1 | PPE: 10.8; PPO: 10.8 | 10 | 130 ml 50% benzene<br>60 ml 20% ethyl ether |
| 2 | SSS: 30.0; PPO: 30.6; SOO: 32.9; OOO: 34.8 | 10 | 90 ml 40% benzene<br>95 ml 60% benzene<br>100 ml 80% benzene<br>100 ml 20% ethyl ether |
| 3 | Mixture of PPO and PPL:50.0 | 8 | 50 ml 40% benzene<br>100 ml 50% benzene<br>100 ml 60% benzene<br>30 ml 20% ethyl ether |
| 4 | Palm oil: 97 | 10 | 100 ml 40% benzene<br>200 ml 60% benzene<br>100 ml 80% benzene<br>120 ml 20% ethyl ether<br>120 ml 100% ethyl ether |

[a] Adsorbent was admixed with filter aid, Celite 535, in a ratio of 2:1.

of *trans* double bonds was carried out by a Unicam SP-200 spectrophotometer with methyl elaidate as the standard.

In run No. 1, fractionation of a mixture of dipalmito-elaidin and dipalmito-olein, the $C_{18}$-monoenoic methyl esters of fractions A and B were isolated by means of gas chromatography and the presence of *trans* double bonds investigated by means of IR spectroscopy.

## Results and Discussion

Figures 1,2,3 and 4 show separations obtained with various synthetic and natural mixtures of triglycerides for which silica gel impregnated with silver nitrate was used as the chromatographic adsorbent. The experimental data show in Table I. The fatty acid analyses of fractions A, B, etc. obtained in the experiments 2,3 and 4 show in Tables II, III and IV, respectively. The percentages of substance recovered varied between 91 and 106%. The analytical results of the experiments 1,2 and 3 show that some of the fractions obtained are contaminated with small amounts (up to 10%) of compounds, which in fact belong to other fractions. All these experiments confirm, however, the great selectivity of the silver nitrate/silica adsorbent and its applicability to the separation of closely related compounds according to number and configuration of the carbon-carbon double bonds. Figure 4 and Table IV show the application of the adsorbent to the fractionation of a natural oil. Palm oil was separated into 6 different fractions. Their degree of unsaturation was 0.06, 1.08, 2.13, 2.04, 3.06 and 4.16 double bonds triglyceride molecule of A,B,C,D,E and F, respectively. The fatty acid compositions of the fractions A,B,C and D correspond closely with those of tripalmitin (PPP), dipalmito-olein (PPO), dipalmito-linolein (PPL) and palmito-diolein (POO), respectively in which P is mainly palmitoyl next to a small amount of other saturated acyl-groups.

TABLE II

Analytical Data of Fractions of Run 2 (Fig. 2)

| Methyl ester (mole %) | A | B | C | D |
|---|---|---|---|---|
| Oleic | 0 | 38 | 62 | 95 |
| Stearic | 96 | 3 | 36 | 4 |
| Palmitic | 4 | 59 | 2 | 1 |
| Recovery (mg) | 28.6 | 31.0 | 33.5 | 30.0 |

Total recovery 123.1 mg = 96% of initial amount

TABLE III

Analytical Data of Fractions of Run 3 (Fig. 3)

| Methyl ester (mol %) | A [a] | B | C |
|---|---|---|---|
| Linoleic | .... | 0 | 32 |
| Oleic | .... | 34 | 2 |
| Stearic | .... | 3 | 0 |
| Palmitic | .... | 63 | 66 |
| Recovery (mg) | 1.7 | 16.1 | 35.1 |

Total recovery 52.9 mg = 106% of initial amount

[a] Fraction A probably contained fully saturated triglycerides.

TABLE IV

Analytical Data of Fractions of Palm Oil (Fig. 4)

| Me-ester mole % | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| $C_{20}$ | 0 | 1 | 2 | 0.5 | trace | trace |
| $C_{18:3}$ | 0 | 0 | 0 | 0 | trace | 3 |
| $C_{18:2}$ | 0 | trace | 34 | 1.5 | 26 | 38 |
| $C_{18:1}$ | 2 | 36 | 3 | 66 | 50 | 31 |
| $C_{18}$ | 10 | 8 | 7 | 4 | 3 | 5 |
| $C_{16}$ | 81 | 52 | 52 | 27 | 20 | 20 |
| $C_{14}$ | 5 | 2 | 2 | 1 | 1 | 1 |
| $C_{12}$ and lower | 2 | 1 | trace | trace | trace | 2 |
| Recovery (mg) | 4.5 | 32.1 | 7.1 | 25.9 | 14.0 | 4.6 |

Total recovery 88.2 mg = 91% of initial amount

Fraction E should contain triglycerides with 3 double bonds. Its fatty acid composition is in agreement with that expected for a mixture containing 75% palmito-oleo-linolein (POL) and 25% triolein (OOO). Fraction F probably contains triglycerides with 4 or more double bonds (PLL, OOL, POL and OLL). A more careful choice of eluants may lead to further separations. The relatively large amount of saturated fatty acid in fraction F may indicate that the polarity of this fraction is partly caused by the presence of a slight amount of oxidized material, possibly formed during chromatography.

The probable triglyceride composition of palm oil, as calculated from the analytical data of Table IV is compiled in Table V. Low-temp crystallization is another separation technique for the determination of triglyceride compositions of natural oils (12,13). Meara (12) applied this method to palm oil and his data (given in Table V) gave no indication of the presence of PPL whereas an amount of 8% was determined by the $AgNO_3$/silica method. It is probable that in Meara's experiments the PPL crystallized, at least in part, with the PPO. By other methods such as crystallization in a thermal gradient (14) and chromatography on a column with silicic acid (15), only partial fractionations have been obtained.

Additional information on the triglyceride composition of an oil can be obtained by oxidation by means of a mixture of periodic acid and permanganate to the corresponding azelaylglycerides (16,17, 18). This method, which determines the amount of triglycerides with respectively zero, one, two or three

TABLE V

Palm Oil Component Triglycerides

| Triglyceride [a] | Method: Crystallization (Meara) | Method: $AgNO_3$-silica (Present work) | Method: Oxidation [b] |
|---|---|---|---|
| PPP | 6 | 5 | 6 |
| PPO, PPL $]P_2U$ | 38, .... ] 38 | 36, 8 ] 44 | ] 44 |
| POO, POL $]PU_2$ | 37, 11 ] 48 | 30, 12 ] 42 | ] 36 |
| OOO, Higher unsat. $]U_3$ | ] 8 | 4, 5 ] 9 | ] 14 |

[a] O = Oleoyl.
L = Linoleoyl.
U = Unsaturated (oleoyl, linoleoyl).
P = Mainly palmitoyl next to a small amount of other saturated acyl-groups.

[b] Experiment was carried out by G. Jurriens (18), Unilever Research Laboratory, Vlaardingen.

unsaturated fatty acid groups, was also applied to our palm oil sample and the results obtained also show in Table V. As can be seen, there is a reasonable agreement between the results of the various analytical methods; however, data for two different samples of palm oil show in Table V.

Since the above chromatographic method constitutes a real fractionation without chemical transformation of the sample, all fractions thus obtained can be subjected to additional analytical investigation. For instance, enzymatic hydrolysis with pancreas lipase, which gives information about the distribution of the fatty acid composition in the $\alpha$- and $\beta$-positions, can be applied.

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# Soybean Unsaponifiables: Hydrocarbons from Deodorizer Condensates

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## Abstract

Molecular distillation of deodorizer condensates followed by chromatography on alumina, gave substantial quantities of hydrocarbons free of other unsaponifiable constituents. Squalene comprised 50% of the hydrocarbon fraction and contained practically all the unsaturation. A crystalline, saturated hydrocarbon fraction of 4.2% was composed primarily of $C_{29}$ and $C_{31}$ paraffins. An unresolved fraction was composed of two major components, each estimated to contain about 30 or 35 carbon atoms. Minor amounts of many hydrocarbons with chain lengths of 15–35 carbon atoms were present but not completely identified. $C^{14}$ analysis showed that the hydrocarbons are natural to soybean oil and they are not artifacts arising from petroleum solvent residues.

## Introduction

SATURATED, UNSATURATED and terpenoid hydrocarbons have been reported as constituents of the unsaponifiable fractions obtained from vegetable oils (1,5,8,10). Hydrocarbons constituted the bulk of unsaponifiable matter in deodorizer distillates obtained from peanut, cottonseed, sunflower and palm oils (8). The even-numbered carbon chains so common to fatty acids are not observed in unsaponifiable hydrocarbons. Nor does the hydrocarbon chain length appear to hold any relationship to the molecular size of the component fatty acids. Aliphatic hydrocarbons from $C_{13}$ to $C_{52}$ have also been reported as unsaponifiable constituents (8,9).

Usually strong odors are not associated with hydrocarbons, but Marcelet (9) reports an agreeable aromatic odor for a $C_{13}H_{24}$ diene hydrocarbon obtained from olive oil and strong odors and a nauseating taste for two unsaturated hydrocarbons obtained from peanut oil. Jasperson and Jones (8) concluded from their examination of deodorizer distillates from six vegetable oils that the predominant type of hydrocarbon was terpenoid, which possessed a strong odor and nauseating taste. Their study did not include soybean oil. However, chromatographic curves published by Cappella et al. (1) indicate that the composition is similar to that of other vegetable oils. Our previous publication indicated that only the hydrocarbon portion of unsaponifiables contributed to the flavor problem of edible soybean oil (7). Hydrocarbons from soybean unsaponifiables have not been thoroughly

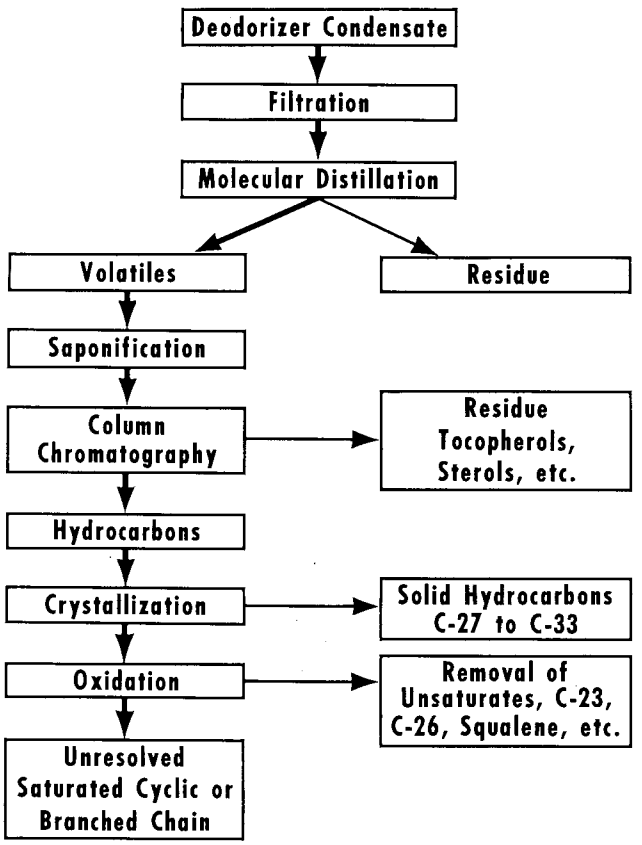


FIG. 1. Flow sheet for fractionation of deodorizer condensate.

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