# The Quantitative Analysis of Triglyceride Mixtures by Thin Layer Chromatography on Silica Impregnated with Silver Nitrate

**C. B. BARRETT, M. S. J. DALLAS, and F. B. PADLEY, Unilever Research Laboratory, Welwyn, Hertfordshire, England**


## Abstract

The paper describes a procedure involving thin layer chromatography on silica impregnated with silver nitrate, which makes possible the separation of synthetic and natural glyceride mixtures into classes according to their degree of unsaturation, and within those classes the resolution of certain isomeric unsaturated glycerides. The separations depend upon the ability of compounds having an olefinic double bond to form co-ordination complexes with the silver ion.

For qualitative analysis, developed chromatoplates are sprayed with dibromofluorescein. Reliable quantitative data may be obtained by charring the separated glycerides with phosphoric acid under carefully controlled conditions, and comparing the densities of the charred areas by means of a photodensitometer. Experiments with mixtures of pure synthetic glycerides revealed an error of less than 3%.

Glyceride compositions of a number of natural fats have been determined, and compared with compositions obtained by enzymic hydrolysis of the same samples.


## Introduction

WHEREAS recent developments, notably in gas-liquid chromatography, have simplified the determination of the fatty acid composition of natural oils, methods for the determination of glyceride composition are less well advanced. Some insight into the glyceride composition of fatty oils has been achieved by the application of such techniques as fractional crystallization (1–6); fractional crystallization after permanganate oxidation (7–9); counter-current distribution (10–11); thermal gradient crystallization (12,13); and column (14), paper (15), and thin layer chromatography (16–19). More detailed information has been calculated from enzymic hydrolysis methods (20–23), either applied directly to the oil, or after fractionation.

In general, fractionation procedures have made possible separations broadly into the four glyceride types: $GS_3$, $GS_2U$, $GSU_2$, and $GU_3$, but have failed to distinguish between isomeric, unsaturated glycerides. Described here is a procedure involving chromatography on thin layers of silica impregnated with silver nitrate, which makes possible the separation of glycerides into classes according to their degree of unsaturation, and within these classes the resolution of certain isomeric unsaturated glycerides.

The separations depend upon the ability of compounds having an olefinic linkage to form co-ordination complexes with silver ions. The complexes formed between the silver ion and a variety of unsaturated hydrocarbons have been investigated extensively by Lucas and co-workers (24–26), mainly by partition studies between an organic (carbon tetrachloride) phase and an aqueous silver nitrate phase. The prediction of Nichols (27) that a distribution of this type might provide a basis for the separation of *cis*- and *trans*-isomers, and in particular methyl *cis*-octadecenoate (oleate) and methyl *trans*-octadecenoate (elaidate) was recently verified by Dutton (28) by means of counter-current distribution between light petroleum and aqueous methanolic silver nitrate. De Vries (29) achieved the same result in a modified solvent system, and by the use of silver nitrate supported on silica, he demonstrated also the separation of certain triglycerides, differing either in total unsaturation or in the steric configuration of the component fatty acids.

In an earlier publication (30) we gave a preliminary account of the qualitative analysis of synthetic and natural glyceride mixtures by thin-layer chromatography (TLC) on silver nitrate impregnated silica. We describe here the development of the technique to a stage where reliable quantitative data may be derived.

## Experimental

*Preparation of plates for qualitative analysis.* Chromatoplates were prepared according to the general method of Stahl (31). Glass plates 20 × 20 cm were coated with a slurry of Silicagel G, Merck, 30 g, in aqueous silver nitrate, 60 ml, 12.5% solution, shaken gently to remove irregularities in the film, and dried at 110C for 60 min. The amount of slurry was regulated to give a silica layer 325 $\mu$ thick.

*Development of the plates.* Glyceride mixtures were applied as solutions in chloroform, 1–3 $\mu$l; 0.5%, along a baseline 2.5 cm from the lower, horizontal edge of the plate. Development was by the ascending technique in a closed tank, 20–24C for 80 min, with a mixture of carbon tetrachloride, 60 vol, chloroform, 40 vol, and acetic acid, 0.5 vol, to which variable small amounts of ethanol were added, according to the type of glycerides to be separated. This subject is elaborated below.

After development the plates were dried in a current of air, and sprayed with 0.2% ethanolic solution of dibromo-R-fluorescein (British Drug Houses Ltd.); the separated glycerides then appeared as bright yellow fluorescent spots when viewed in ultra-violet light.

*Preparation of chromatoplates for quantitative analysis.* To obtain reliable quantitative data, certain modifications were made of the described procedure, and certain precautions were observed.

Thin glass plates, 20 × 20 × 0.2 cm, were used, and were roughened on the face to be coated by rubbing on a second glass plate sprinkled with water and carborundum powder. The silica adhered strongly to the ground surface, and showed less tendency to bubble during the subsequent charring of the spots. The adsorbent was applied to the plates in the manner al-

ready described; dust particles, cloth fibers, and other foreign matter were rigorously excluded.

Chloroform solutions of the glycerides, 2 $\mu$l, 0.5%, were applied at intervals of 2.2 cm along a baseline 2.5 cm from the lower horizontal edge of the plate, considerable care being exercised not to damage the silica film at the point of application.

Chromatoplates were developed at 20–24C for 80 min in closed tanks by the ascending technique in the solvent mixture already described.

After development the plates were dried in air, and cut into strips 2.2 cm wide, each strip being one chromatogram. Cutting was carried out by scoring with a diamond glass cutter, using a ruler as a guide; cleavage of the thin glass plates occurred either spontaneously, or after gentle tapping along the scored line. The strips were sprayed uniformly with sufficient aqueous orthophosphoric acid, 50%, to just wet the silica, dried for 2 min above an aluminum block heated to 340 C, and finally placed upon the block for 5 min.

Densities of the charred areas were measured on the "Chromoscan" photodensitometer (Joyce Loebl and Co., Gateshead, England. Instrument adjusted to specimen/record ratio 3:1, aperture 10 × 1 mm, light grey filter). For any one glyceride, the area under the densitometer curve was proportional to the quantity of glyceride, but areas were influenced by the type of glyceride, and also to some extent by variations in the thickness of the silica layer. To compensate for these factors, a typical chromatoplate consisted of seven chromatograms, three of the test mixture, and four of a standard mixture consisting of equal amounts of pure triglycerides, arranged alternately. The manner in which the corrections were applied is described below.

## Results and Discussion

In the initial exploratory work, which involved the use of plates coated with mixtures of silver nitrate impregnated silica and hexane/diethyl ether as developing solvent, a good degree of resolution of mixtures of synthetic mono-acid and mixed stearic-oleo acid type triglycerides was obtained, mainly according to iodine value. Under similar conditions, no significant resolution was obtained on plates which did not incorporate silver nitrate.

On silver nitrate/silica plates, 2-oleodistearin consistently showed a higher Rf value than 1-oleodistearin, although because of tailing of the spots, mixtures of these glycerides were not clearly resolved. The observations indicated, however, that mobility was influenced not only by total unsaturation, but by the arrangement of the fatty acids within the triglyceride molecule.

A variety of developing solvents were then examined. The inclusion of small amounts of acetic acid had the effect of improving definition, and typical separations achieved with a solvent consisitng of 99.5% chloroform and 0.5% acetic acid are shown diagrammatically in Figure 1. Clear separations, according to iodine value, of triglycerides (Column A), diglycerides and monoglycerides (Column C) were obtained, and the isomeric glycerides also resolved include 1- and 2-oleodistearin, 1- and 2-linoleo-distearin, and 1,2 and 1,3 diolein.

More generally useful however, particularly in the analysis of natural fats, was a developing solvent consisting of carbon tetrachloride, 60 vol, chloroform, 40 vol, and acetic acid, 0.5 vol, to which variable small amounts of ethanol were added. In a solvent mixture containing about 0.4% of ethanol, trisaturated glycerides, e.g., tristearin, had an Rf value of about 0.9 and clear separations of glycerides having up to three double bonds were obtained at the expense of lack of definition among the more unsaturated types. Increase in the amount of ethanol in the developing

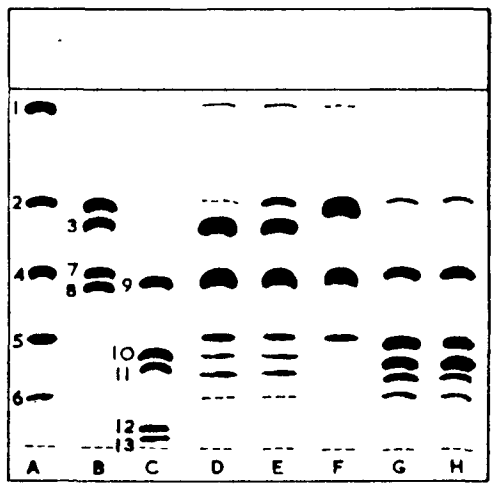


FIG. 1. Thin layer chromatography of glycerides and natural fats on a silica gel G—silver nitrate adsorbent. A,B,C, synthetic glyceride mixtures; D, lard; E, interesterified lard; F, cocoa butter; G, cottonseed oil; H, groundnut oil. I, Tristearin; 2, 2-oleodistearin; 3, 1-oleodistearin; 4, 1-stearodiolein; 5, triolein; 6, trilinolein; 7, 2-linoleodistearin; 8, 1-linoleodistearin; 9, 1,3-distearin; 10, 1,2-diolein; 11, 1,3-diolein; 12, monostearin; 13, monoolein.

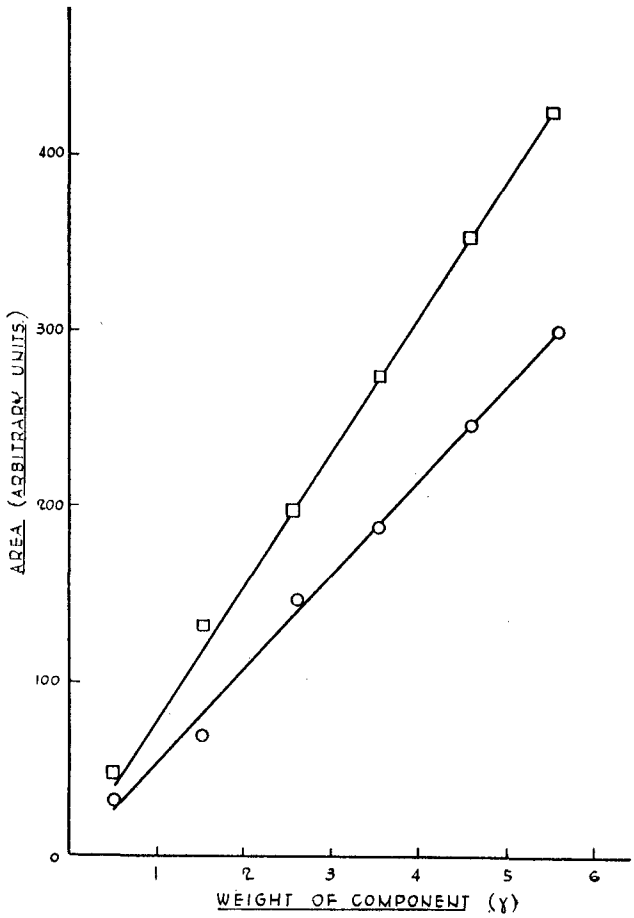


FIG. 2. Graph of weight of glyceride charred against area under densitometer curve. □ = Tristearin. ○ = Triolein.

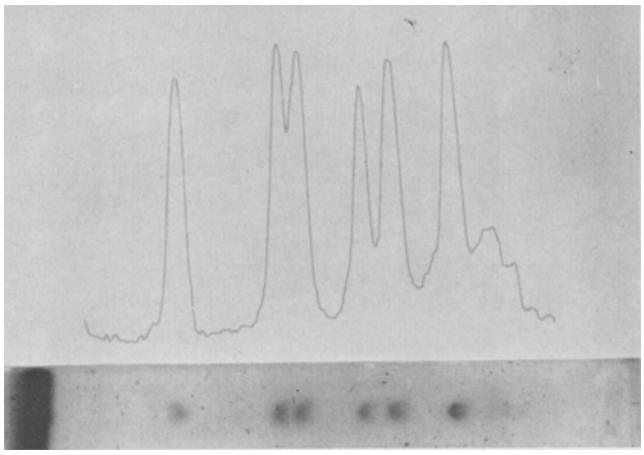

FIG. 3. Chromatogram and densitometer curve of mixture of equal amounts of tristearin; 1- and 2-oleodistearin, 2-linoleodistearin, 1-stearodiolein, and triolein.

solvent caused a general increase in Rf value; at a level of 1–1.5% the Rf value of triolein was of the order of 0.9, and improved resolution of the more unsaturated glycerides was obtained.

For qualitative analysis of glyceride mixtures by this general TLC procedure, dibromofluorescein was found to be the most suitable spray reagent for the detection of spots. The separated glycerides were not readily detected by iodine vapor, rhodamine B, or Sudan Black. Although no precise quantitative data could be gained in this way, some estimate of contamination of, e.g., synthetic 2-oleodistearin by 1-oleodistearin was readily obtained. Gross contamination, $> 1\%$, was detectable by a single chromatography. Trace contamination, $< 1\%$, was detected by chromatography of a band of the suspect mixture, cutting out and extracting the area corresponding to the position of 1-oleodistearin and rechromatographing as a single spot.

Quantitative analysis of more complex glyceride mixtures was made possible by a procedure similar in principle to that described by Privett and Blank (32), who sprayed the developed chromatoplates with 50% aqueous sulfuric acid, charred, and measured the relative densities of the charred areas. On silica/silver nitrate plates, saturated triglycerides were not appreciably attacked by sulfuric acid, but for various glycerides including saturated triglycerides, more nearly uniform charring was obtained after spraying with phosphoric acid, the use of which for this purpose has not hitherto been reported.

The relative densities of the charred spots were measured by means of a reflectance densitometer. Areas under the densitometer curves were related linearly to the amount of glyceride charred (Fig. 2), but were influenced by the type of glyceride (Figs. 2,3,4) and to some extent also by the thickness of the silica layer. For accurate determination of glyceride composition corrections for each of these factors were applied.

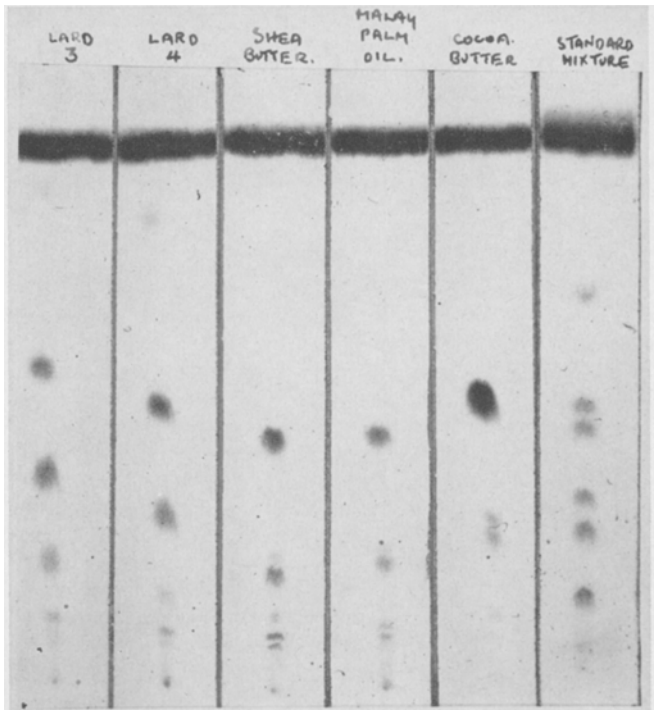


FIG. 4. Thin-layer chromatograms of lard, shea, and cocoa butters, and palm oil.

Errors due to variation of the thickness of the silica layer were small and were considerably reduced by taking averages of several determinations of composition. Compensation for the influence of glyceride type was made by chromatographing, alongside the test sample, a mixture of equal amounts of pure triglycerides. The area under the densitometer curve corresponding to one component, e.g., triolein, was expressed as unity, and the areas corresponding to the others calculated as multiples or sub-multiples of this area. The reciprocals of the figures so obtained became the correction factors by which the appropriate areas under the densitometer curve for the test sample were multiplied. The derivation of typical correction factors is illustrated in Table I.

In practice, one chromatoplate contained seven chromatograms, three of the test sample and four of the standard mixture, arranged alternately. The composition of the test mixture was calculated from each chromatogram and corrected on the basis of the mean correction factors derived from the flanking chromatograms of the test mixture. The true composition was taken then as the average of these three determinations.

The accuracy of this procedure may be judged from analyses (Table II) of mixtures of pure glycerides of varying composition; in each case the correction factors were calculated from chromatograms of a mixture of equal amounts of tristearin, 1- and 2-oleodistearin, 1-stearodiolein, and triolein. The error is almost invariably less than 3%.

TABLE I

Calculation of Correction Factors (CF) from Chromatograms of the Standard Mixture

| Order on chromatoplate | 1 Standard mixture | | Mean CF (Mean 1+3) | 3 Standard mixture | | Mean CF (Mean 3+5) | 5 Standard mixture | | Mean CF (Mean 5+7) | 7 Standard mixture | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Component | Area | CF | | Area | CF | | Area | CF | | Area | CF |
| Triolein | 215 | 1.00 | 1.00 | 200 | 1.00 | 1.00 | 165 | 1.00 | 1.00 | 159 | 1.00 |
| 1-Stearodiolein | 225 | 0.96 | 0.97 | 205 | 0.98 | 0.89 | 209 | 0.79 | 0.75 | 226 | 0.70 |
| 1-Oleodistearin | 398 | 0.57 | 0.62 | 301 | 0.66 | 0.58 | 328 | 0.50 | 0.51 | 313 | 0.51 |
| 2-Oleodistearin | 350 | 0.61 | 0.68 | 272 | 0.74 | 0.65 | 298 | 0.55 | 0.51 | 349 | 0.47 |
| Tristearin | 623 | 0.35 | 0.39 | 472 | 0.43 | 0.37 | 540 | 0.31 | 0.32 | 488 | 0.33 |

TABLE II

Analysis of Triglyceride Mixtures of Known Composition

| Mixture | A | | B | | C | | D | | E | | F | | G | | H | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Component | Known | Found | Known | Found | Known | Found | Known | Found | Known | Found | Known | Found | Known | Found | Known | Found |
| Triolein | 6.5 | 8.5 | 6.5 | 12.0 | 13.5 | 19.0 | 33.5 | 35.0 | 20.0 | 18.5 | 26.5 | 26.0 | 15.0 | 14.5 | 10.0 | 10.5 |
| 1-Palmitodiolein | 33.5 | 33.5 | 33.5 | 31.5 | 6.5 | 6.0 | 26.5 | 27.0 | 13.5 | 14.0 | 20.0 | 17.0 | 50.0 | 49.0 | 40.0 | 43.0 |
| 1-Oleodistearin | 26.5 | 25.5 | 26.5 | 25.5 | 33.5 | 32.0 | 20.0 | 21.5 | 6.5 | 4.0 | 13.5 | 12.0 | 25.0 | 29.0 | 35.0 | 34.5 |
| 2-Oleodistearin | 20.0 | 18.0 | 20.0 | 19.5 | 26.5 | 24.0 | 13.5 | 12.5 | 33.5 | 33.0 | 6.5 | 4.5 | 5.0 | 4.0 | 5.0 | 2.5 |
| Tristearin | 13.5 | 15.0 | 13.5 | 11.5 | 20.0 | 19.0 | 6.5 | 4.0 | 26.5 | 28.5 | 33.5 | 37.0 | 5.0 | 3.5 | 10.0 | 9.5 |

TABLE III

Glyceride Compositions of Natural Fats by TLC and Enzymic Hydrolysis

| Analyses | Cocoa butter | | Shea butter | | Malayan Palm | | Lard 1 | | Lard 2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Component | Enzymic hydrolysis | TLC | EH | TLC | EH | TLC | EH | TLC | EH | TLC |
| SSS | 3.0 | ..... | 1.5 | Trace | 10.0 | 8.0 | 4.5 | Trace | 12.0 | 4.0 |
| SOS | 80.0 | 73.5 | 41.5 | 43.0 | 32.5 | 35.5 | 1.0 | 1.5 | 1.5 | 1.5 |
| OSS | ..... | ..... | ..... | ..... | 8.0 | 3.0 | 26.0 | 24.5 | 39.5 | 35.0 |
| S lin S | 6.0 | 6.0 | 7.5 | 9.0 | 6.5 | 8.0 | ..... | ..... | ..... | ..... |
| Lin SS | ..... | ..... | ..... | ..... | 1.0 | ..... | 48.5 | 47.0 | 39.0 | 42.5 |
| SOO + OSO | 10.0 | 17.0 | 32.0 | 29.0 | 26.0 | 29.0 | ..... | ..... | ..... | ..... |
| SO lin + OOO | 1.0 | 3.5 | 14.5 | 13.0 | 13.0 | 9.0 | 18.0 | 22.0 | 7.0 | 14.5 |
| GU[4] | ..... | ..... | 2.5 | 3.0 | 3.0 | 7.5 | 2.5 | 5.0 | 0.5 | 4.0 |
| GU[5] | ..... | ..... | Trace | 3.0 | ..... | ..... | ..... | ..... | ..... | ..... |

S = saturated fatty acid.
O = monounsaturated fatty acid.
Lin = di-unsaturated fatty acid.
GU[4], GU[5] = glycerides with 4 or 5 double bonds.

TABLE IV

Fatty Acid Compositions of Natural Oils Examined by Enzymic Hydrolysis

| | Cocoa butter | | Shea butter | | Malayan palm oil | | Lard 1 | | Lard 2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Triglyceride | Monoglyceride | Triglyceride | Monoglyceride | Triglyceride | Monoglyceride | Triglyceride | Monoglyceride | Triglyceride | Monoglyceride |
| $C_{10}$ | ..... | ..... | ..... | ..... | ..... | ..... | ..... | Trace | ..... | ..... |
| $C_{12}$ | ..... | ..... | ..... | ..... | Trace | 0.5 | ..... | 0.25 | ..... | ..... |
| $C_{14}$ | ..... | ..... | ..... | ..... | 1.0 | 1.0 | 1.5 | 4.0 | 1.5 | 3.5 |
| $C_{16}$ | 27.0 | 1.5 | 3.4 | ..... | 45.5 | 16.5 | 27.0 | 71.25 | 32.0 | 78.0 |
| $C_{16-2H}$ | ..... | ..... | 1.1 | ..... | 0.5 | 0.5 | 4.0 | 6.5 | 3.5 | 3.75 |
| $C_{17}$ | ..... | ..... | 0.7 | ..... | ..... | ..... | Trace | 0.5 | 0.5 | 0.5 |
| $C_{18}$ | 36.0 | 1.75 | 41.1 | 3.5 | 4.5 | 1.5 | 11.5 | 3.0 | 17.5 | 4.75 |
| $C_{18-2H}$ | 34.5 | 89.75 | 47.1 | 81.4 | 41.5 | 67.0 | 51.0 | 13.75 | 43.5 | 9.5 |
| $C_{18-4H}$ | 2.5 | 7.0 | 6.6 | 15.1 | 7.0 | 13.0 | 5.0 | 0.75 | 1.5 | ..... |

Applications of this TLC procedure to the analysis of oils and fats are illustrated by the chromatogram of cocoa and shea butters, palm oil, and lard (Fig. 4). These fats have a low content of the more unsaturated (linoleo-) glycerides, and hence a satisfactory analysis was obtained by chromatography in one solvent mixture only. Calculated glyceride compositions are tabulated (Table III), and compared with compositions determined from data obtained by enzymic hydrolysis of the same samples. Fatty acid compositions of the fats, and of the monoglycerides after enzymic hydrolysis are given in Table IV.

The improved resolution made possible by variation of the amount of alcohol in the solvent mixture is exemplified by the chromatograms (Fig. 5) of soybean, cottonseed, and peanut oils. Calculated glyceride compositions, and comparative data from enzymic hydrolysis, for the peanut and cottonseed oils are collected in Table V. In the case of soybean oil, lack of a suitable range of pure linoleic and linolenic acid glycerides has prevented a complete quantitative analysis.

The results demonstrate good agreement between glyceride compositions determined by TLC and enzymic hydrolysis, although some discrepancy between the proportions of fully saturated glycerides is to be noted. Glyceride compositions were determined from enzymic hydrolysis data by the method of Coleman and Fulton (33), which involves experimental determination of the acids esterified at the secondary glycerol hydroxyl group, and random distribution of the remaining acids at the primary hydroxyl groups. By comparison, TLC has the merit that glycerides

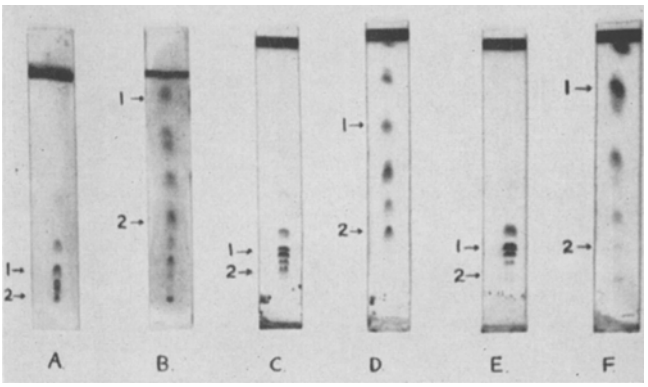


FIG. 5. Thin layer chromatograms of soybean, cottonseed, and peanut oils. A, B = Soybean oil. C, D = Cottonseed oil. E, F = Peanut oil. 1 = Triolein. 2 = Trilinolein. A,C,E, developed at 20C with chloroform, 30 vol, carbon tetrachloride, 70 vol, ethanol, 0.5 vol, acetic acid, 0.5 vol. B,D,F, developing solvent as for A,C,E, but containing ethanol, 1.5 vol.

TABLE V

Glyceride Compositions of Natural Oils by TLC and Enzymic Hydrolysis

| Component | Peanut oil | | Cottonseed oil | |
|---|---|---|---|---|
| | Enzymic hydrolysis | TLC | Enzymic hydrolysis | TLC |
| SOS | 4.0 | 3.0 | 7.5 | 5.5 |
| SOO + S lin S | 24.5 | 26.0 | 21.0 | 22.5 |
| OOO + SO lin | 46.5 | 39.0 | 18.5 | 22.5 |
| GU[4] | 21.5 | 23.5 | 27.0 | 30.0 |
| GU[5] | 3.5 | 7.5 | 13.0 | 12.5 |
| GU[6] | ..... | ..... | 15.0 | 6.5 |

S = saturated fatty acid.
O = mono-unsaturated fatty acid.
Lin = di-unsaturated fatty acid.
GU[4], GU[5], GU[6] = glycerides containing 4, 5 or 6 double bonds.

are determined directly, and that in calculations of glyceride composition, no theories of acyl group distribution need to be invoked.

The interpretation of chromatograms of oils such as coconut and palm kernel is complicated by the influence of the chain lengths of the esterified acids on the Rf values of the constituent glycerides. For those fats for which we quote data here, the component fatty acids are predominantly palmitic and steric acids or their unsaturated analogues, and no fractionation which could be attributed to differences in chain length was observed. In the case of the nut oils, the differences in fatty acid chain length are much greater, and exert some noticeable influence on the Rf values of the component glycerides.

ACKNOWLEDGMENT

Experimental work during this investigation by Miss M. McMullin and C. R. Gauterin.

REFERENCES

1. Quimby, D. T., R. L. Wille, and E. S. Lutton, JAOCS, *30*, 186 (1953).
2. Riemenschneider, R. W., C. E. Swift, and C. E. Sands, Oil and Soap, *17*, 145 (1940).
3. Riemenschneider, R. W., JAOCS, *31*, 266 (1954).
4. Riemenschneider, R. W., F. E. Luddy, M. L. Swain, and W. C. Ault, Oil and Soap, *23*, 276 (1946).
5. Brown, J. B., Chem. Revs., *29*, 333 (1941).
6. Hilditch, T. P., The Chemical Constitution of the Natural Fats, 2nd Ed., Wiley, New York, 1947.
7. Hilditch, T. P., and C. H. Lee, J. Chem. Soc., 3106 (1927).
8. Kartha, A. R. S., JAOCS, *30*, 326 (1953).
9. von Rudloff, E., Can. J. Chem., *34*, 1413 (1956).
10. Dutton, H. J., and J. A. Cannon, JAOCS, *33*, 46 (1956).
11. Dutton, H. J., C. R. Scholfield, and T. L. Mounts, *Ibid.*, *38*, 96 (1961).
12. Magnusson, J. R., E. G. Hammond, *Ibid.*, *36*, 325 (1959).
13. Jones, G. V., and E. G. Hammond, *Ibid.*, *38*, 96 (1961).
14. Sahasrabudhe, M. R., and D. G. Chapman, *Ibid.*, *38*, 88 (1961).
15. Inouye, Y., and M. Noda, J. Agric. Chem. Soc. Japan *33*, 452 (1959).
16. Kaufmann, H. P., Fette-Seifen Anstrichmittel, *63*, 807 (1961).
17. Kaufmann, H. P., *Ibid.*, *63*, 828 (1961).
18. Kaufmann, H. P., Z. Makus, and T. H. Khoe, *Ibid.*, *64*, 1 (1962).
19. Michalec, C., M. Sule, and J. Mistan, Nature, *193*, 63 (1962).
20. Mattson, F. H., and L. W. Beck, J. Biol. Chem., *219*, 735 (1956).
21. Savary, P., and P. Desnuelle, Biochem. Biophysica. Acta, *21*, 349 (1956).
22. Coleman, M. H., *JAOCS*, *38*, 685 (1961).
23. Youngs, C. G., *Ibid.*, *38*, 62 (1961).
24. Lucas, H. J., and W. F. Eberz, J. Am. Chem. Soc., *56*, 460 (1934).
25. Eberz, W. F., M. J. Welge, D. M. Yost, and H. J. Lucas, *Ibid.*, *59*, 45 (1937).
26. Winstein, S., and H. J. Lucas, *Ibid.*, *60*, 836 (1938).
27. Nichols, P. L., *Ibid.*, *74*, 1091 (1952).
28. Dutton, H. J., C. R. Scholfield, and E. P. Jones, Chem. & Ind., 1874 (1961).
29. deVries, B., Paper presented to the VIth I.S.F.Congress, London, 1962, Chem. & Ind. 1049 (1962).
30. Barrett, C. B., M. S. J. Dallas, and F. B. Padley, Chem. & Ind., 1050 (1962).
31. Stahl, E., Pharmazie, *11*, 633 (1956).
32. Privett, O. S., and M. L. Blank, J. Lipid Research *2*, 37 (1961).
33. Coleman, M. H., and W. G. Fulton, Int. Conf. Biochem. Problems of Lipids, Pergamon Press, London (1961).

[Received November 5, 1962—Accepted April 12, 1963]

# Methods for Improving Yields of Cyclic Acid from Linseed Oil[1]

**J. P. FRIEDRICH, J. C. PALMER, E. W. BELL, and J. C. COWAN, Northern Regional Research Laboratory,[2] Peoria, Illinois**

## Abstract

Liquid C-18 saturated monocarboxylic acids, which are termed "cylic acids" because they contain a ring structure, have been prepared by the action of excess sodium hydroxide on linseed oil in ethylene glycol at elevated temperatures, followed by distillation and hydrogenation of the resulting free fatty acid monomers and by separation of the straight-chain components by low-temperature crystallization from acetone. In a survey of other possible catalysts and reaction conditions, cyclic acid yields were improved from the previously reported 32.4 g to 43.5 g of cyclic acid per 100 g of linseed fatty acids by removing water from the starting materials and using the monosodium derivative of ethylene glycol as catalyst. The corresponding amount of polymer formed decreased because of a decrease in hydroxylation and subsequent polyester formation.

## Introduction

EARLIER C-18 saturated monocarboxylic acids that fail to crystallize at −70C were reported to have been prepared from linseed oil, linolenic acid, and tung oil (3). The relative proportions of liquid cyclic acids, straight-chain monomeric acids, and polymer formed varied with the substrate and the conditions employed. Under reported conditions, an appreciable loss in total acid equivalents occurred during distillation of crude acids, as indicated by the carboxyl balance. Subsequent investigations showed that the polymers had neutralization equivalents ranging from 450–1,100. Saponification of these polymers reduced neutralization equivalents to about 300, only slightly higher than expected for normal polymeric acids. Esterification and subsequent distillation of the saponified fraction yielded additional cyclic and other monomeric acids, as well as a residue of polymeric acid having a neutralization equivalent slightly above 300.

In this latest work, removing water from the reaction mixture and using the monosodium derivative of ethylene glycol as catalyst increased yields of cyclic acids from a previously reported 32.4 g (3)–43.5 g/100 g of linseed fatty acids, with correspondingly less hydroxylation and subsequent polyester formation.

## Experimental

*Preparation of Catalyst-Solvent Systems.* Except for the nickel catalyst, systems were prepared by dissolving a catalyst in an appropriate amount of solvent to produce a concentration such that a 1:3 (wt/vol) linseed fatty acid to solvent ratio would give a 50% excess of catalyst.

Ethylene glycol-sodium hydroxide: 60 g (1.5 moles) of reagent grade NaOH was dissolved in 834 ml of stock ethylene glycol.

Dry ethylene glycol-sodium hydroxide: made by dissolving 60 g of reagent grade NaOH in 834 ml of ethylene glycol which had been distilled from $\frac{1}{20}$ its weight of Na. A constant boiling fraction was collected at 196C.

Ethylene glycol-monosodium derivative of ethylene glycol: made by dissolving 34.5 g of Na in 870 ml of stock ethylene glycol with vigorous stirring. Tempera-

[1] Presented at the AOCS meeting, in Chicago, Ill., 1961.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, U.S.D.A.