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# DETECTION OF ADULTERATION OF BUTTER FAT (GHEE) BY THE RANDOM REARRANGEMENT REACTION AND THIN-LAYER CHROMATOGRAPHY

M. M. CHAKRABARTY, D. BHATTACHARYYA AND A. K. GAYEN
*Department of Applied Chemistry, University Colleges of Science and Technology, Calcutta University, Calcutta-9 (India)*

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## SUMMARY

A new method, involving the use of the effect of the random rearrangement reaction in fats has been developed which detects 5–10% (w/w) of adulterants such as hydrogenated groundnut, tallow and mohua (Mowrah) fats in butter fat (ghee). The method consists of the isolation of the trisaturated glycerides ($GS_3$) of rearranged pure and adulterated ghee by silver nitrate–silica gel thin-layer chromatography, and separation of the isolated $GS_3$ into individual glyceride components by reversed phase chromatography on liquid paraffin coated thin layers of Kieselguhr G using acetone–methanol–acetic acid (60:40:0.5) as developing solvent. Some $GS_3$ components of ghee increase after rearrangement and the presence of the above adulterants further increases their concentration. Tallow and mohua (Mowrah) fats increase the concentration of the $GS_3$ components of butter fat more than hydrogenated groundnut fat after randomisation. A prominent difference in the occurrence of the fatty acids, principally $C_{12}$ to $C_{16}$, also exists between some $GS_3$ components of rearranged pure butter fat (ghee) and rearranged adulterated butter fats.

When both hydrogenated groundnut and mohua (Mowrah) fats are adulterants, the $C_{12}$ to $C_{16}$ acids of some $GS_3$ components of pure butter fat become more concentrated after the random rearrangement. With tallow as adulterant, however, the concentration of the $C_{12}$ to $C_{16}$ acids in some $GS_3$ components having similar mobility compared to pure butter fat significantly decreases after random rearrangement.

Variations in the concentrations of the trisaturated glyceride components, including constituent fatty acids, between pure butter fat and adulterated butter fats are better visualised when the fats are randomly rearranged than without rearrangement.

## INTRODUCTION

One type of rearrangement reaction in glycerides involves the inter and intra molecular exchange of acyl radicals of the glycerides, with or without a catalyst at suitable temperatures. When a triglyceride mixture (natural or synthetic) is subjected

to rearrangement, a mixture of glycerides is formed in which the distribution of the acyl groups is statistical or random and the overall glyceride composition of the rearranged products differs from the original combination. The alteration in glyceride composition of some natural oils after random rearrangement was readily detected, with the help of TLC, by CHAKRABARTY *et al.*[1,2] and by PRIVETT *et al.*[3]. CHAKRABARTY *et al.* have also suggested that the rearrangement reaction involving the randomisation principle may be utilised for detecting adulteration of an oil (fat with other oils) or fat by considering the changes in pattern that are likely to occur with respect to the difference in number of component glycerides and their concentration in the pure and adulterated glyceride oils before and after random rearrangement. The use of the rearrangement reaction for detecting groundnut oil in mustard oil, in conjunction with TLC, has been reported by CHAKRABARTY *et al.*[4].

The present paper describes the detection of adulterants such as hydrogenated groundnut, tallow and mohua (Mowrah) fats in butter fats (ghee) at the 5–10% level by first conducting the random rearrangement reaction and then adopting the TLC technique. It should be stated that these adulterants have been chosen for a comparison of the efficacy of the present method with an earlier report[5] by some of us and for the extension of our research on the development of methods for detection of adulteration in oils and fats some of which have been reported[5,6].

## EXPERIMENTAL

### *Random rearrangement reaction and isolation of the rearranged products*

The method adopted was essentially that of CHAKRABARTY *et al.*[7]. Pure butter fat (ghee) and butter fat (ghee) containing 5–10% (w/w) of fats like hydrogenated groundnut, mohua (Mowrah) and tallow were dissolved separately in *n*-hexane so as to form a 60% solution (w/w) and agitated by a magnetic stirrer with 0.4% sodium methoxide (based on the weight of the fat solution) for 30 min in a small conical flask. The catalyst was destroyed by 1:3 HCl and the products were taken up in ether and the ether layer was washed free of HCl by distilled water. The ether solution, after drying over anhydrous sodium sulphate, was filtered and the ether removed in nitrogen atmosphere. The fats were purified from methyl esters of mono- and diglycerides that might be present in the rearranged fats by preparative adsorption silica gel TLC.

### *Isolation of the trisaturated glycerides ($GS_3$) from pure and randomly rearranged fats*

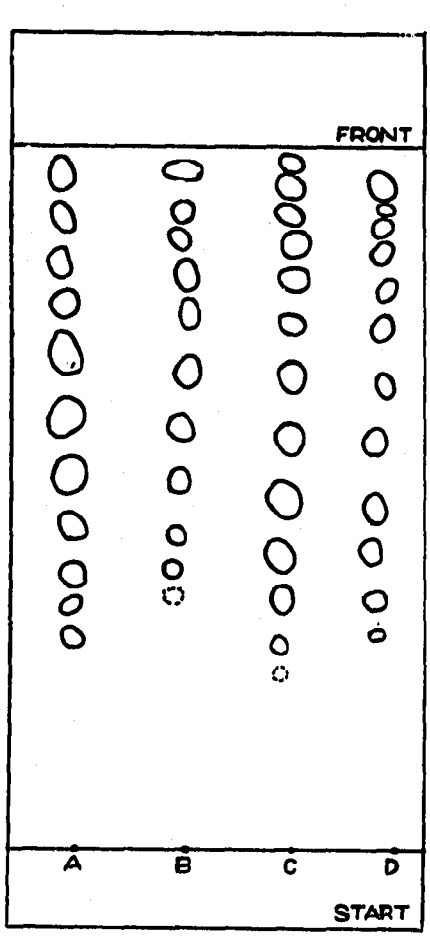
$GS_3$ was isolated from identical quantities of pure and adulterated randomised products, according to the method of CHAKRABARTY *et al.*[5], by elution with $CHCl_3$ containing 0.5% acetic acid from $AgNO_3$–Silica Gel G TLC plates.

### *Separation of total glycerides and trisaturated glyceride ($GS_3$) components of pure and adulterated randomised and unrandomised butter fat (ghee) samples*

Separation was achieved by eluting twice with a solvent system consisting of acetone–methanol–acetic acid (60:40:0.5) on liquid paraffin impregnated Kieselguhr G thin-layer plates, and the glyceride components were detected as blue violet spots by iodine vapour followed by a spray of a 2% solution of starch in 20% ethanol[5].

*Identification of the component fatty acids in some individual trisaturated glycerides ($GS_3$) of fats (ghee) both before and after rearrangement*

Some $GS_3$ components having identical positions on the chromatograms but differing in concentration were scraped from plates and saponified with 2 *N* methanolic KOH and extracted with petroleum ether (40–60°) to remove paraffin. They were then acidified with 1:3 HCl and extracted again with diethyl ether. After washing 2 to 3 times with a few millilitres of water the ether was removed in nitrogen atmosphere and the fatty acids left were weighed and dissolved in benzene to give 1% solutions. The benzene solutions were then applied, in the form of spots, to a paraffin impregnated Kieselguhr G layer and eluted with acetic acid (90%) saturated with liquid paraffin. The spots were detected as before by iodine vapour and starch solution.



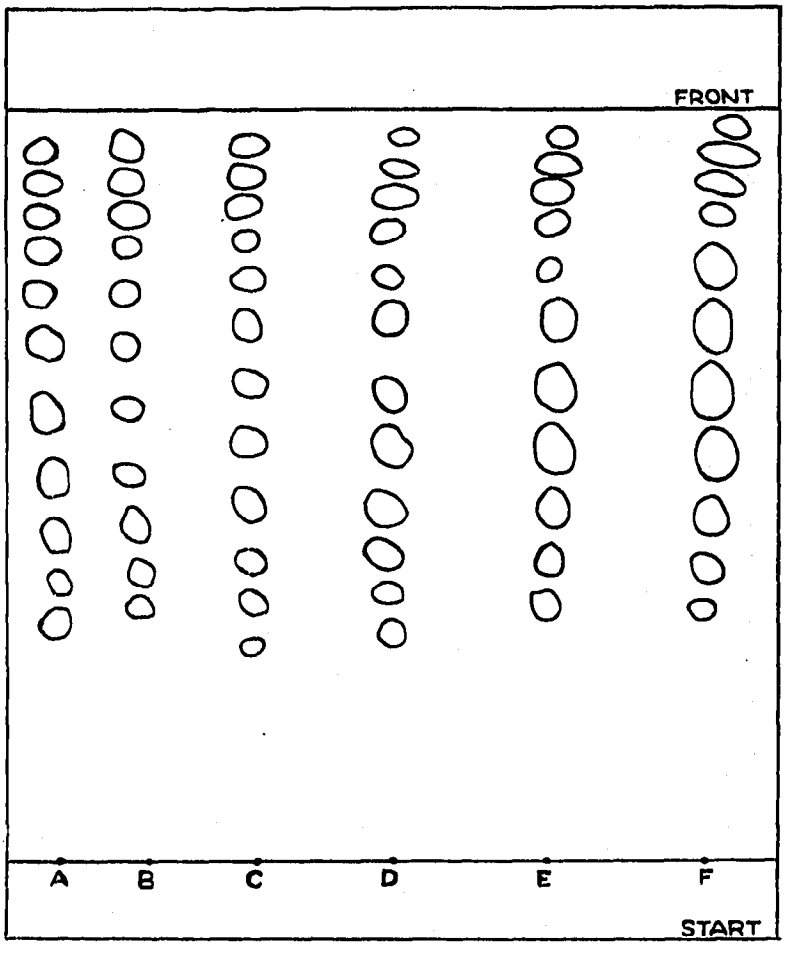




Fig. 1. Separation of total glycerides. (Total amount spotted, 60 μg). A = butter fat (ghee); B = randomised butter fat (ghee); C = butter fat adulterated with 5% hydrogenated groundnut fat before randomisation; D = butter fat adulterated with 5% hydrogenated groundnut fat after randomisation.

Fig. 2. Separation of total glycerides. (Total amount spotted, 60 μg.) A = butter fat (ghee); B = randomised butter fat (ghee); C = butter fat adulterated with 5% mohua (Mowrah) fat before randomisation; D = butter fat adulterated with 5% mohua (Mowrah) fat after randomisation; E = butter fat adulterated with 5% tallow before randomisation; F = butter fat adulterated with 5% tallow after randomisation.

TABLE I

CHARACTERISTICS OF THE SAMPLES INVESTIGATED

Sap. value = saponification value, R.I. = refractive index, R.M. = Reichert–Meissl value, R.P. = Reichert–Polenske value.

| *Sample* | *Iodine value* | *Sap. value* | *R.I. at 40°* | *R.M.* | *R.P.* | *Slip point (°C)* |
|---|---|---|---|---|---|---|
| 1 Butter fat (Ghee) | 31.0 | 222.0 | 1.4533 | 30.2 | 1.6 | 28.5 |
| 2 Randomised butter fat (ghee)[a] | — | — | — | — | — | 31.5 |
| 3 Hydrogenated groundnut fat | 56.1 | 185.1 | 1.4594 | — | — | 41.0 |
| 4 Randomised hydrogenated groundnut fat[a] | — | — | — | — | — | 36.0 |
| 5 Mohua (Mowrah) fat | 60.5 | 190.0 | 1.4600 | — | — | 22.0 |
| 6 Randomised mohua (Mowrah) fat[a] | — | — | — | — | — | 31.0 |
| 7 Tallow | 44.3 | 196.5 | 1.4583 | — | — | 49.0 |
| 8 Randomised tallow[a] | — | — | — | — | — | 46.5 |
| 9 Butter fat adulterated with 5% hydrogenated groundnut fat before randomisation | 32.4 | 220.8 | 1.4554 | 28.2 | 1.5 | 30.0 |
| 10 Butter fat adulterated with 5% hydrogenated groundnut fat after randomisation[a] | — | — | — | — | — | 32.0 |
| 11 Butter fat adulterated with 10% hydrogenated groundnut fat before randomisation | 33.6 | 218.5 | 1.4559 | 26.3 | 1.4 | 30.5 |
| 12 Butter fat adulterated with 10% hydrogenated groundnut fat after randomisation[a] | — | — | — | — | — | 32.0 |
| 13 Butter fat adulterated with 5% mohua (Mowrah) fat before randomisation | 32.2 | 220.4 | 1.4605 | 28.7 | 1.4 | 29.0 |
| 14 Butter fat adulterated with 5% mohua (Mowrah) fat after randomisation[a] | — | — | — | — | — | 32.0 |
| 15 Butter fat adulterated with 10% mohua (Mowrah) fat before randomisation | 33.7 | 218.8 | 1.4608 | 27.6 | 1.4 | 29.5 |
| 16 Butter fat adulterated with 10% mohua(Mowrah) fat after randomisation[a] | — | — | — | — | — | 30.5 |
| 17 Butter fat adulterated with 5% tallow before randomisation | 31.8 | 221.0 | 1.4558 | 28.4 | 1.4 | 29.0 |
| 18 Butter fat adulterated with 5% tallow after randomisation[a] | — | — | — | — | — | 35.5 |
| 19 Butter fat adulterated with 10% tallow before randomisation | 32.6 | 219.7 | 1.4561 | 26.5 | 1.4 | 29.5 |
| 20 Butter fat adulterated with 10% tallow after randomisation[a] | — | — | — | — | — | 35.5 |

[a] The analytical characteristics, excepting slip point ,were not determined because such characteristics generally remained unaltered after the rearrangement reaction.

## RESULTS

The slip points of the fats before and after randomisation by the A.O.C.S.[8] method are indicated in Table I.

The chromatographic separations of the total glycerides of pure and adulterated

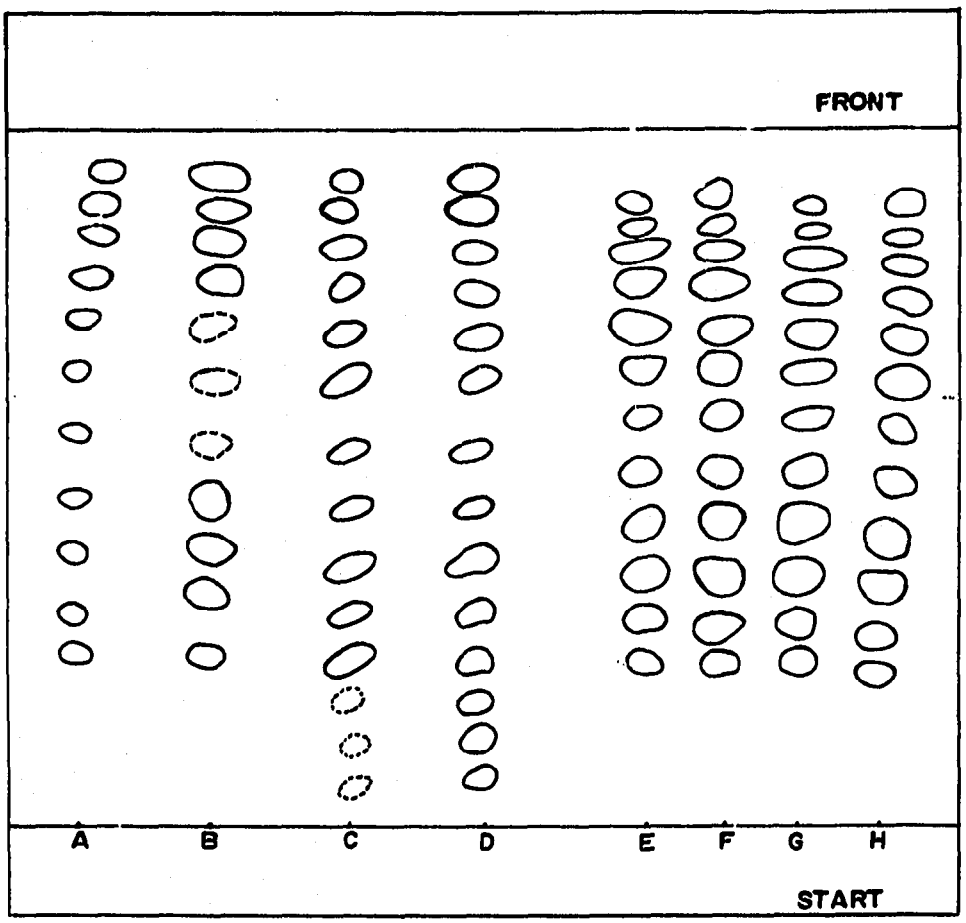



Fig. 3. Separation of trisaturated glycerides. (Total amount spotted, 80 $\mu$g.) A = butter fat (ghee); B = randomised butter fat (ghee); C = butter fat adulterated with 5% hydrogenated groundnut fat before randomisation; D = butter fat adulterated with 5% hydrogenated groundnut fat after randomisation; E = butter fat adulterated with 5% mohua (Mowrah) fat before randomisation; F = butter fat adulterated with 5% mohua (Mowrah) fat after randomisation; G = butter fat adulterated with 5% tallow before randomisation; H = butter fat adulterated with 5% tallow after randomisation.

butter fat (ghee) samples before and after the randomisation reaction are shown in Figs. 1 and 2.

The chromatogram of the trisaturated glycerides of pure and adulterated butter fats before and after random rearrangement is shown in Fig. 3.

The relative concentrations of the fatty acids present in the selected trisaturated glyceride components of pure butter fat and butter fat adulterated with the hydrogenated groundnut, tallow and mohua (Mowrah) fats separated by reverse phase TLC are shown in Table II (A–D). The + sign in these tables indicates the approximate concentration. An increased number of + signs denotes increased concentration. Separations of fatty acids by TLC are shown in Figs. 4–7.

## DISCUSSION

The chromatographic separations of total glycerides of butter fat (ghee) and adulterated butter fats (*vide* Figs. 1 and 2) indicate the influence of the random

E II

ATIVE DETECTION OF COMPONENT FATTY ACIDS

| *s of trisaturated glycerides* | *Fatty acids* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_4$ | $C_6$ | $C_8$ | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ |
| *action 11 numbered from top* | | | | | | | | | | |
| utter fat (ghee) | | | | + + + | + + + | + + + | + + + | + + + | | |
| ndomised butter fat (ghee) | | | | + + | + + | + + | + + | + | | |
| utter fat (ghee) adulterated th 5% hydrogenated oundnut fat before randomisation | | | | + + + | + + + | + + + | + + + | + + | | |
| utter fat (ghee) adulterated th 5% hydrogenated oundnut fat after randomisation | | | | + + + + | + + + + | + + + + | + + + + | + | | |
| utter fat (ghee) adulterated th 5% mohua (Mowrah) before randomisation | | | | + | + | + + | + + | | | |
| utter fat (ghee) adulterated th 5% mohua (Mowrah) after randomisation | | | | + | + | + | + | | | |
| utter fat (ghee) adulterated th 5% tallow, before randomisation | | | | + | + | + | + | + | | |
| utter fat (ghee) adulterated th 5% tallow, after randomisation | | | | + + + + | + + + + | + + + + | + + + + | | | |
| *action 10 numbered from the top* | | | | | | | | | | |
| utter fat (ghee) | | | | + + | + + | + + | + + | + | | |
| ndomised butter fat hee) | | | | + + | + + | + + + | + + | + | | |
| utter fat (ghee) adulterated th 5% hydrogenated oundnut fat before randomization | | | | + | + | + + | + + | + | | |
| utter fat (ghee) adulterated th 5% hydrogenated oundnut fat after randomization | | | | + + + | + + + | + + + + | + + + + | + + | + | |
| utter fat (ghee) adulterated th 5% mohua (Mowrah) t before randomisation | | | | + | + | + + | + | + | | |
| utter fat (ghee) adulterated th 5% mohua (Mowrah) t after randomisation | | | | + | + | + + + + | + | + | | |
| utter fat (ghee) adulterated th 5% tallow before randomisation | | | | + + | + + + | + + | + + | | | |
| utter fat (ghee) adulterated th 5% tallow after randomization | | | | + | + | + | + | | | |
| *action 4 numbered from e top* | | | | | | | | | | |
| utter fat (ghee) | | | | + + + | + + + | + + + | + + + | | | |
| andomised butter fat (ghee) | | | | + + + + | + + + + | + + + + | + + + + | + + | | |
| utter fat (ghee) adulterated th 5% hydrogenated oundnut fat before randomization | | | | + | + | + | + | | | |

*(continued on p. 122)*

TABLE II *(continued)*

| *Samples of trisaturated glycerides* | *Fatty acids* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_4$ | $C_6$ | $C_8$ | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ |
| Butter fat (ghee) adulterated with 5% hydrogenated groundnut fat after randomisation | | | | | + + + + + | + + | + + + + + + | + + | |
| Butter fat (ghee) adulterated with 5% mohua (Mowrah) fat before randomisation | | | | | + | + | + | | |
| Butter fat (ghee) adulterated with 5% mohua (Mowrah) fat after randomisation | | | | + | + + | + + | + + | | |
| Butter fat (ghee) adulterated with 5% tallow before randomisation | | | | + | + | + + + + | + + + + | | |
| Butter fat (ghee) adulterated with 5% tallow after randomisation | | | | | | + | + | | |
| *(D) Fraction 3 numbered from the top* | | | | | | | | | |
| Butter fat (ghee) | | | | + + | + + + | + + + | + + + | + + + | |
| Randomised butter fat (ghee) | | | | + | + + | + + | + + | + + | |
| Butter fat (ghee) adulterated with 5% hydrogenated groundnut fat before randomisation | | | | + | + + | + + | + + | + + | |
| Butter fat (ghee) adulterated with 5% hydrogenated groundnut fat after randomisation | | | | | + | + | + | + | |
| Butter fat (ghee) adulterated with 5% mohua (Mowrah) fat before randomisation | | | | + | + | + | + | + | |
| Butter fat (ghee) adulterated with 5% mohua (Mowrah) fat after randomisation | | | | | + + + + | + + + + | + + + + | + + + + | |
| Butter fat (ghee) adulterated with 5% tallow before randomisation | | | | + + | + + + | + + + | + + + | + + + | |
| Butter fat (ghee) adulterated with 5% tallow after randomisation | | | | + | + | + | + | + | |

rearrangement reaction on the alteration in the composition of glycerides. The concentrations of some of the constituent glycerides of pure butter fat increase when adulterated with hydrogenated groundnut, mohua (Mowrah) and tallow when compared with pure butter fat after randomisation. This observation suggests the presence of adulterants in butter fat.

However, commensurate with our previous observations[5] the detection of adulteration becomes easier and more conclusive when the trisaturated glycerides are first isolated from the pure and adulterated butter fats before and after randomisation and then resolved into their components by reversed phase TLC.

The concentration of some of the $GS_3$ components of pure butter fat and adulterated butter fats increases after randomisation. It is also interesting to note

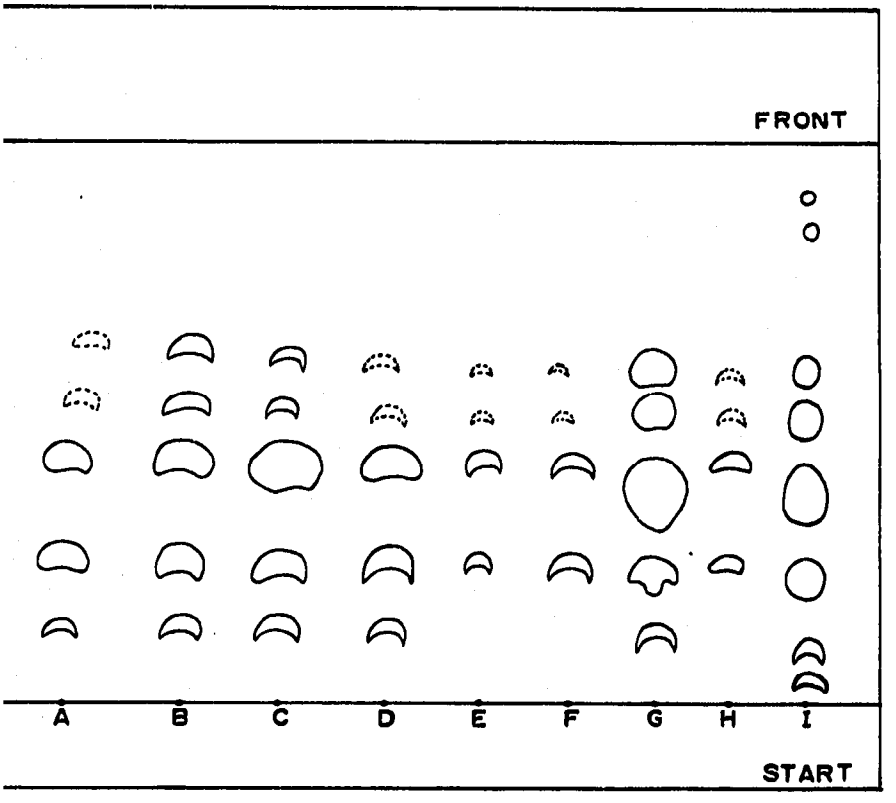



Fig. 4. Separation of fatty acids of trisaturated glycerides (fraction 11 from the top). (Total amount spotted, 30 $\mu$g.) A = pure butter fat (ghee); B = randomised butter fat (ghee); C = ghee adulterated with 5% hydrogenated groundnut fat before randomisation; D = ghee adulterated with 5% hydrogenated groundnut fat after randomisation; E = ghee adulterated with 5% mohua oil before randomisation; F = ghee adulterated with 5% mohua oil after randomisation; G = ghee adulterated with 5% tallow before randomisation; H = ghee adulterated with 5% tallow after randomisation; I = standard fatty acid mixture ($C_6$–$C_{22}$).

hat some $GS_3$ components of the adulterated rearranged fats are more concentrated han the corresponding $GS_3$ components of randomised pure butter fat and unrandomsed adulterated butter fats. The increase in concentration, however, depends on the ype of adulterant. Thus tallow, on account of its inherently typical glyceride composition, and mohua (Mowrah) fat, because of the higher quantity of saturated fatty icids ($C_{16}$ to $C_{18}$) compared with hydrogenated groundnut fat, markedly increase the concentration of trisaturated glyceride components of randomised butter fat having dentical mobilities. The increase in concentration contributed by the hydrogenated groundnut fat is less, presumably owing to the lower content of total saturated fatty icids, notably $C_{16}$, compared with tallow and mohua and also due to the presence of *trans*-oleic acids which may behave differently from the other two fats during randomisation. The pronounced difference between the pure randomised butter fat and the adulterated randomised butter fats, with respect to the concentration of their trisaturated glyceride spots, allows the rapid detection of adulterants in butter fat.

The identification of fatty acids of some selected $GS_3$ components of pure butter fat and adulterated fats, both before and after randomisation, reveals some nteresting features (*vide* Table II).

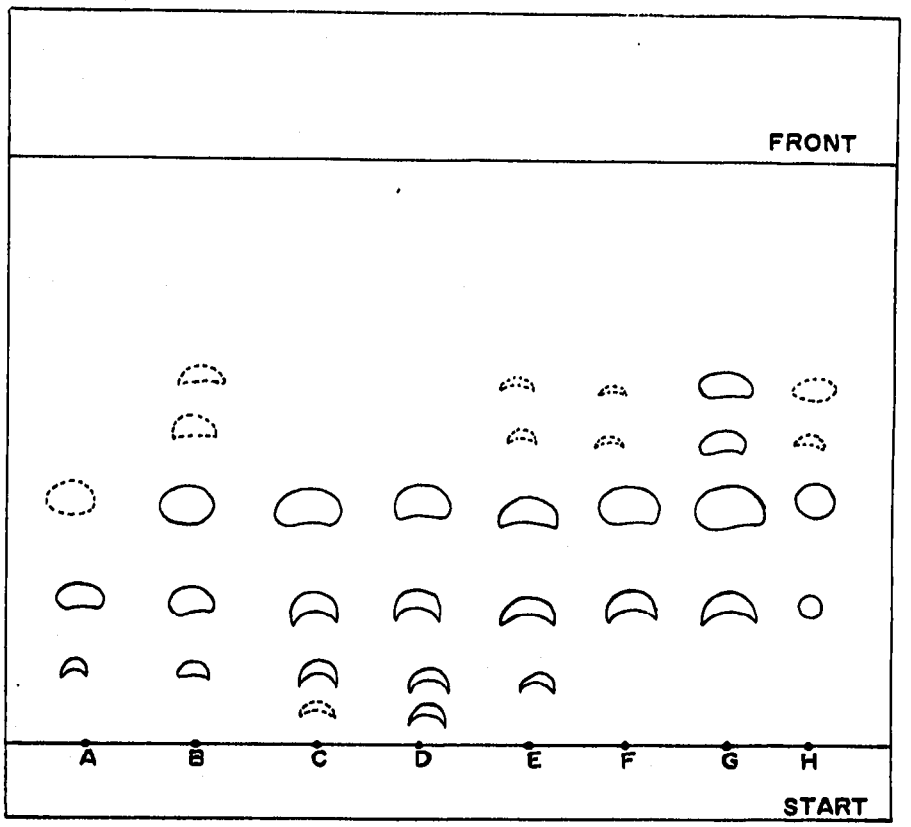



Fig. 5. Separation of fatty acids of trisaturated glycerides (fraction 10 from the top). (Total amount spotted, 30 $\mu$g.) A–H are as in Fig. 4.

The presence of $C_8$ to $C_{20}$ fatty acids and their relative concentrations again depends on the nature of the fats. Thus in the trisaturated glycerides ($GS_3$) (fraction 11 numbered from the top) of the samples it appears that $C_{10}$ to $C_{16}$ acids of randomised ghee are less concentrated than pure ghee. But when ghee contains hydrogenated groundnut fat and tallow as adulterants, the $C_{10}$ to $C_{16}$ fatty acids become more concentrated than in ghee after randomisation. Before randomisation the fatty acid content of ghee containing hydrogenated groundnut fat is similar to that of unrandomised ghee and the $C_{10}$ to $C_{16}$ fatty acids in the case of ghee containing tallow are less concentrated than in both ghee and randomised ghee. On the other hand, the concentration of $C_{10}$ to $C_{16}$ fatty acids in ghee containing mohua (Mowrah) fat before and after randomisation is much less than in ghee and randomised ghee.

Similarly in the trisaturated glyceride fractions (numbered 10 from the top) some difference in the concentrations of the fatty acids is noted. Thus $C_{10}$ to $C_{18}$ fatty acids of ghee containing hydrogenated groundnut fat after randomisation are observed to be more concentrated than in ghee, randomised ghee and an unrandomised mixture of ghee and hydrogenated groundnut fat. Ghee containing tallow after randomisation shows lower amounts of $C_{10}$ to $C_{16}$ fatty acids than ghee, randomised ghee and an unrandomised blend of ghee and tallow. Furthermore, $C_{20}$ fatty acid has been detected in randomised ghee containing hydrogenated groundnut fat. In the case of ghee adulterated with mohua, it should be noted that after randomisation

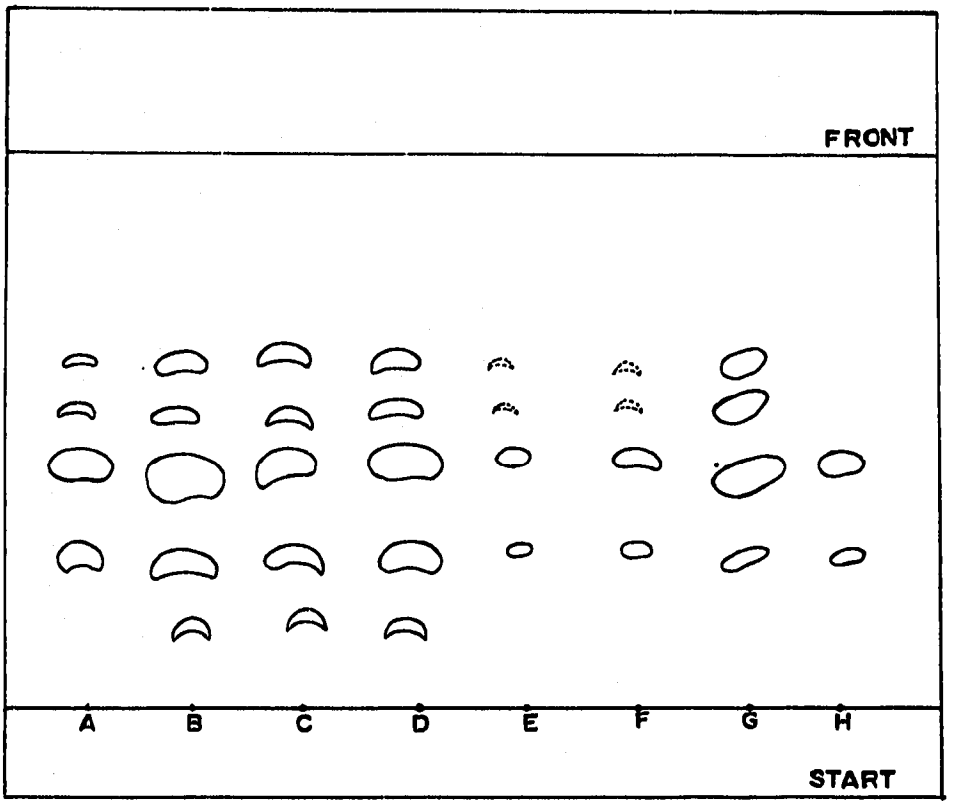



Fig. 6. Separation of fatty acids of trisaturated glycerides (fraction 4 from the top). (Total amount spotted, 30 $\mu$g.) A–H are as in Fig. 4.

only the $C_{14}$ fatty acid becomes more concentrated than in the unrandomised mixture, ghee and randomised ghee.

The trisaturated glyceride fraction (numbered 4 from the top) of ghee shows that the concentration of $C_{10}$ to $C_{16}$ fatty acids is much less than the corresponding fraction of randomised ghee which, in addition to the above acids, contains $C_{18}$ fatty acid. In the trisaturated glyceride (fraction 4 from the top) of ghee containing hydrogenated groundnut fat the $C_{10}$ fatty acid is not detected after randomisation and there is less $C_{14}$ fatty acid than in ghee but more $C_{16}$ fatty acid than in randomised ghee and ghee. Before randomisation the presence of tallow in ghee is found to increase the amounts of $C_{14}$ and $C_{16}$ fatty acids of ghee. After randomisation the amounts of the said acids are remarkably less compared with those of randomised ghee and ghee; the $C_{10}$ and $C_{12}$ fatty acids were also not detectable. Ghee containing mohua shows $C_{10}$ to $C_{16}$ fatty acids in greater amounts after randomisation than in the unrandomised mixture but less than in ghee and randomised ghee.

$C_{10}$ to $C_{18}$ fatty acids of the trisaturated glyceride component of ghee (numbered 3 from the top) are more concentrated than the corresponding fatty acids of the trisaturated glycerides of randomised ghee. Ghee adulterated with hydrogenated groundnut fat indicates that there are less $C_{10}$ to $C_{18}$ acids than those in ghee but they are almost similar in concentration to those of randomised ghee. After randomisation of ghee containing hydrogenated groundnut fat $C_{12}$ to $C_{18}$ fatty acids become much less concentrated than even in randomised ghee. Ghee containing mohua after

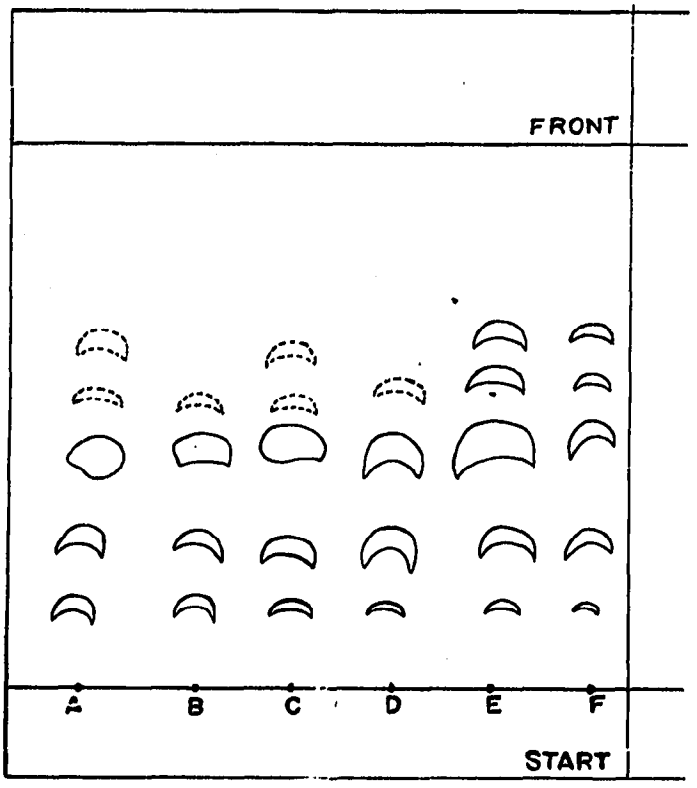



Fig. 7. Separation of fatty acids of trisaturated glycerides (fraction 3 from the top). (Total amount spotted, 30 $\mu$g.) A = ghee adulterated with 5% hydrogenated groundnut fat before randomisation; B = ghee adulterated with 5% hydrogenated groundnut fat after randomisation; C = ghee adulterated with 5% mohua before randomisation; D = ghee adulterated with 5% mohua after randomisation; E = ghee adulterated with 5% tallow before randomisation; F = ghee adulterated with 5% tallow after randomisation.

randomisation contains principally $C_{12}$ to $C_{18}$ fatty acids in greater amounts than in randomised ghee. But after randomisation the same acids become less concentrated than in ghee, randomised ghee and ghee containing mohua. The $C_{10}$ to $C_{18}$ fatty acids of ghee containing tallow appear to be more concentrated before randomisation than those of randomised ghee. Thus the variation in concentration of some component fatty acids of the trisaturated glyceride spots of ghee and adulterated ghee before and after randomisation corroborate further the detection of adulterants like hydrogenated groundnut fat, mohua (Mowrah) and tallow in ghee.

In this connection it is also important to note that while slip points (*vide* Table I) fail to distinguish pure butter fat from butter fats containing 5 to 10% hydrogenated groundnut fat and mohua after randomisation, the TLC separation of glycerides and the fatty acids thereof, is capable of detecting the influence of the rearrangement reaction on alterations in the glyceride pattern of the fats and consequently facilitates the identification of adulterants in butter fats (ghee).

The combination of the rearrangement reaction and TLC appears, therefore, to be a convenient method for the detection of adulterants in butter fat (ghee) and it can be inferred that the method is likely to be useful in the detection of adulteration in other fats. It is also possible to visualise the possibility of quantitative evaluation of the various glycerides separated, or of fatty acids thereof, by combining selective

enzymatic hydrolysis, gas–liquid chromatography, spectroscopy or other methods with TLC. Such attempts are under way by the present authors.

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