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DETECTION OF ADULTERATION OF FATS BY THIN LAYER CHROMATOGRAPHY OF TRISATURATED GLYCERIDES

I. DETECTION OF HYDROGENATED GROUNDNUT OIL, TALLOW AND MOHUA (MOWRAH) OIL IN BUTTER FAT(GHEE)

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SUMMARY

A new method based on the difference in the number of components and intensities has been developed for the detection of adulteration of buffer fat (Ghee) with high melting point fats such as hydrogenated groundnut oil, tallow and Mohua (Mowrah) oil, by first separating the trisaturated glycerides (GS_3) by argentation-thin layer chromatography on Silica Gel G, then resolving the GS_3 into components on paraffin impregnated Kieselguhr G plates followed by microsaponification. After this the fatty acids are isolated on paraffin coated Kieselguhr G plates to indicate the component fatty acids of particular glyceride spots. The solvent systems used were (i) acetone–methanol–acetic acid (60:40:0.5) for the triglycerides and (ii) 90% acetic acid for fatty acids. Unequivocal detection of adulterants up to 5% level was possible by this method, whereas the conventional methods, depending on physical or chemical characteristics such as refractive index, Reichert-Meissl, Polenske, saponification and iodine values, failed.

INTRODUCTION

Butter fats (ghee) are characterised normally by their Reichert–Meissl, Polenske and Kirschner values. But skilful adulteration of these fats with other closely related fats in minor quantities does not significantly alter the above values and thus may permit such adulterated fats to pass for pure materials. Development of a simple rapid method for detecting such adulteration of buffer fat with other fats like tallow, hydrogenated fats and Mohua (Mowrah) oil, which are the usual adulterants, therefore has considerable importance. The technique of thin layer chromatography has already found use in ascertaining the purity of oils and fats in edible and inedible fields[1–3]. In the present investigation this technique has been adopted for the examination of butter fat and the detection of adulterants in it. The adulterant (beef tallow, hydrogenated groundnut oil and Mohua oil) was added in different minor proportions by weight of the butter fat.

The detection is based on: (i) the chromatographic resolution of the trisaturated glyceride (GS_3) of pure butter fat and adulterated butter fat and (ii) the detection of fatty acids comprising the different components of the trisaturated glyceride.

This method was considered feasible on the following grounds:

(i) Some of the trisaturated glycerides of butter fat are likely to have different mobilities from some of the trisaturated glycerides of beef tallow, hydrogenated groundnut oil, and Mohua oil because of the presence of considerable percentages of low molecular weight fatty acids.

(ii) The component fatty acids of some of the glycerides are likely to differ substantially, for unequivocal indication of the source of adulteration.

These advantages are not immediately apparent when the whole fats are chromatographed, because of the proximity of the R_F values of a greater number of the glycerides present in such mixtures.

In the present study the trisaturated glycerides of pure and adulterated butter fat samples were first isolated by preparative thin layer chromatography on silver nitrate impregnated Silica Gel G plates. The GS_3 (trisaturated glycerides) from each sample were then resolved into components by reversed phase chromatography on a paraffin impregnated Kieselguhr G layer. Each individual component of the GS_3 thus separated was later subjected to microsaponification and the fatty acids detected by separation on paraffin-coated Kieselguhr G layer.

EXPERIMENTAL

Isolation of GS_3 from pure butter fat and butter fat containing 5% and 10% of beef tallow, hydrogenated groundnut and Mohua oil respectively by silver nitrate–Silica Gel G thin layer chromatography, and subsequent fractionation of the isolated GS_3 on a paraffin impregnated kieselguhr layer was done by essentially following the methods of CHAKRABARTY *et al.*[4,5].

Silver nitrate–Silica Gel G plates (20 × 20 cm) of *ca.* 0.5 mm thickness were prepared by spreading a slurry made of 16 g Silica Gel G (E. Merck) and 32 ml of 12.5% aqueous silver nitrate (E. Merck) solution. This was allowed to set in air for 30 min and then baked at 110° for 1½ h. The plates thus prepared were first washed with the eluting solvent by the ascending technique. The plates were later activated for 15 min at 110° before spotting. About 100 mg of butter fat and adulterated butter fat samples (5% solution in benzene, E. Merck) were applied in the form of bands on three such plates and eluted with 99.5 ml chloroform (E. Merck) containing 0.5 ml acetic acid. After elution, each plate was sprayed with an 0.02% solution of sodium fluorescein (E. Merck) and viewed under U.V. light whereupon the yellow fluorescent band of trisaturated glycerides (GS_3) appearing at the top (near the solvent front) was marked and scraped off. The GS_3 band was then extracted repeatedly from the plate by dry diethyl ether. The diethyl ether solution was then passed through a small column of silica gel to remove the indicator following the method of LITCHFIELD *et al.*[6]. The ether solution from the column was collected and the ether removed in an atmosphere of nitrogen in order to obtain the pure "GS_3". GS_3 from each sample was then converted to a definite percent (2%) solution in benzene (E. Merck) before further separation on paraffin coated Kieselguhr G plates.

Kieselguhr G plates (20 × 20 cm) of 0.5 mm thickness were prepared by pouring a slurry of 16 g Kieselguhr G (E. Merck) in 32 ml of distilled water, allowing it to set for 15 min and then baking at 110° for 1 h.

The Kieselguhr G plates were impregnated with a 5% solution of liquid paraffin (b.p., B.D.H.) in petroleum ether (b.p. 40–60°) and left in air for 10 min to remove petroleum ether.

30–60 γ of GS_3 of butter fat and adulterated butter fat samples were spotted and the plate was eluted three times with a solvent system consisting of 60 vol. acetone (A.R.), 40 vol. methanol (E. Merck) and 0.5 vol. acetic acid (A.R.). The solvent was removed by heating the plate for 15 min in an oven at 110°. The saturated triglyceride spots were then detected either by spraying with 0.005% Rhodamine B in 50% ethanol followed by examination under U.V. lamp, or by exposing the plate for 15 min to iodine vapour followed by immediate thorough spraying of 2% starch in 20% ethanol whereby they became visible as blue-violet spots against a light violet background. The chromatograms at the 5% level of adulteration are shown in Fig. 1. This must be considered the limit for the moment, as below this level of adulteration the detection is unsatisfactory.

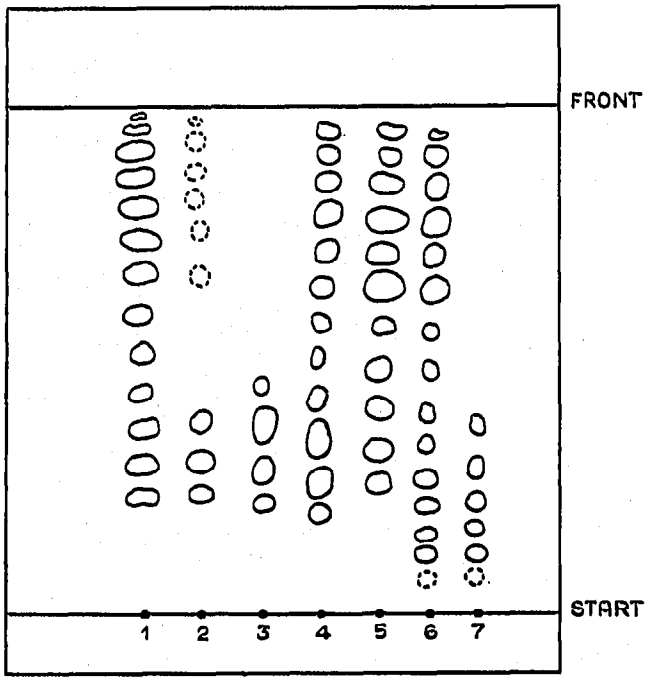

Fig. 1. Thin layer chromatography of trisaturated glycerides (GS_3) on a Kieselguhr G plate impregnated with 5% liquid paraffin. (1) GS_3 of butter fat adulterated with 5% Mohua oil; (2) GS_3 of Mohua oil; (3) GS_3 of tallow; (4) GS_3 of butter fat adulterated with 5% tallow; (5) GS_3 of butter fat; (6) GS_3 of butter fat adulterated with 5% hydrogenated groundnut oil; (7) GS_3 of groundnut oil.

In addition 30–60 γ of butter fat and butter fat containing 10% adulterants such as hydrogenated groundnut oil, tallow and Mohua oil were similarly chromatographed on liquid paraffin impregnated Kieselguhr G plates as described above. Fig. 2 illustrates the chromatogram.

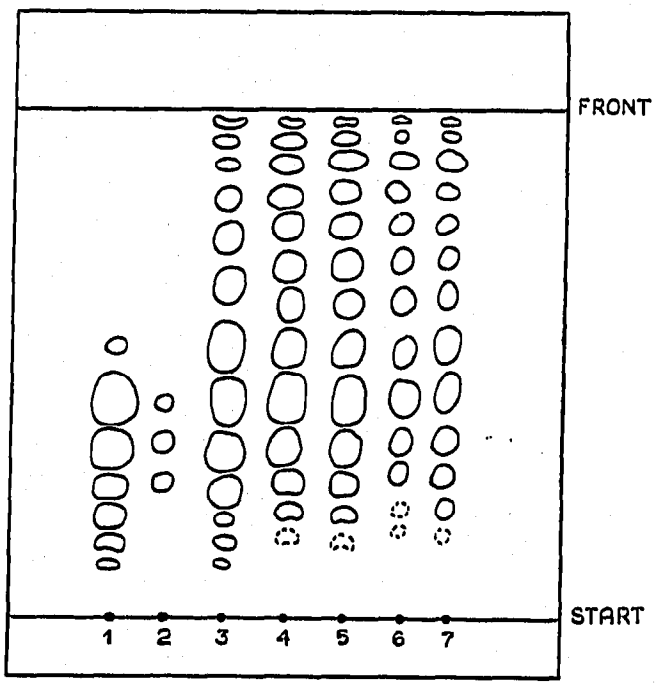

Fig. 2. Thin layer chromatography of glycerides on a Kieselguhr G plate impregnated with 5% liquid paraffin. (1) Groundnut oil; (2) tallow; (3) butter fat adulterated with 5% hydrogenated groundnut oil; (4) butter fat adulterated with 5% Mohua oil; (5) butter fat adulterated with 5% tallow; (6) butter fat; (7) Mohua oil.

Afterwards larger quantities of GS_3 of butter fat and butter fat adulterated with tallow, hydrogenated groundnut oil and Mohua oil were applied in the form of bands on three such plates and eluted thrice with the same eluting solvent. The saturated triglyceride components thus separated in the form of bands were located by spraying only the two edges of the plate with Rhodamine B solution. Corresponding bands on the unsprayed portion under investigation were then scraped off and extracted thrice with diethyl ether. The ether was removed and the material was saponified with a few millilitres of 2 *N* methanolic KOH at room temperature overnight, the methanol was removed by evaporation, the liquid paraffin was then removed by petroleum ether, the soap acidified with 2 *N* HCl and the liberated fatty acids were extracted again with diethyl ether. The ether was removed and the fatty acids taken up in 0.1 ml benzene (E. Merck) and the benzene solution was then applied in the form of a spot on a paraffin-coated Kieselguhr G thin layer plate. The fatty acids were eluted with 90% acetic acid (A.R.) saturated with liquid paraffin.

The fatty acids were detected by comparing them with those of standards by exposing the plate, after removal of acetic acid, to iodine vapour for 10 min, followed by an immediate spray of a 2% solution of starch in 20% ethanol when blue-violet spots against a light violet background appeared.

The presence of the various fatty acids and their relative concentrations are given in Table I.

Some physicochemical characteristics of butter fat and adulterated butter fat samples determined by standard methods[7] are represented in Table II.

TABLE I

QUALITATIVE DETECTION OF COMPONENT FATTY ACIDS PRESENT IN SOME SELECTED TRISATURATED GLYCERIDE BANDS OF PURE BUTTER FAT AND BUTTER FAT ADULTERATED WITH HYDROGENATED GROUNDNUT OIL, TALLOW AND MOHUA (MOWRAH) OIL SEPARATED BY REVERSED PHASE THIN LAYER CHROMATOGRAPHY AS REPRESENTED IN FIG. 1

The '+' sign denotes approximate concentration. An increased number of '+' signs denotes increased concentration.

Samples of trisaturated glycerides	*Band No. numerated from above*	*Fatty acids*										
		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Butter fat	7				+		+	+	+			
	8				++	+	+	+				
	9					+	+	++	+			
	10						+	++	+			
	11						++	++	+			
Butter fat + hydrogenated groundnut oil	9					+	++	++	+			
	12						++	++	++	+	+	
	13						+	++	++	+	+	
	14						+	++	+	+	++	
	15							++	+	+	+	+
Butter fat + tallow	7				+		+	+	+			
	8				+		+	++	+			
	9						+	++	+			
	10							++	++			
	11						+	++	+			
	12							++	++			
Butter fat + Mohua oil	9						+	++	+			
	10							++	++			
	11						+	+	++			
	12							++	++			

TABLE II

CHARACTERISTICS OF SAMPLES INVESTIGATED

Sample	*Iodine value*	*Sap. value*	*R.I. at 40°*	*R.M.*	*R.P.*
1. Butter fat	31.0	222.0	1.4553	30.2	1.6
2. Hydrogenated groundnut oil	56.1	185.1	1.4594	—	—
3. Tallow	44.3	196.5	1.4583	—	—
4. Mohua (Mowrah) oil	60.5	190.0	1.4600	—	—
5. Butter fat adulterated with 5% hydrogenated groundnut oil	32.4	220.8	1.4554	28.2	1.5
6. Butter fat adulterated with 10% hydrogenated groundnut oil	33.6	218.5	1.4559	26.3	1.4
7. Butter fat adulterated with 5% tallow	31.8	221.0	1.4558	28.4	1.4
8. Butter fat adulterated with 10% tallow	32.6	219.7	1.4561	26.5	1.4
9. Butter fat adulterated with 5% Mohua oil	32.2	220.4	1.4605	28.7	1.4
10. Butter fat adulterated with 10% Mohua oil	33.7	218.8	1.4608	27.6	1.4

Sap. value = saponification value; R.I. = refractive index; R.M. = Reichert-Meissl value; R.P. = Reichert-Polenske value.

DISCUSSION

It is clear from the chromatogram shown in Fig. 1 that the GS_3 of the adulterated butter fat has a higher number of individual components compared to pure butter fat. The excess number, however, depends on the type of adulterant. Thus with hydrogenated groundnut oil as adulterant as many as four extra component glycerides are obtained, whereas with beef tallow and Mohua oil there is only one extra glyceride spot below the last glyceride spot of butter fat. In addition to the separation of extra glyceride spots, a marked difference in intensity between the individual trisaturated glyceride spots of the pure and adulterated butter fats is observed.

Separation of fatty acids of triglyceride spots reveals that the four extra GS_3 components obtained in the case of the hydrogenated groundnut oil–butter fat blend contain acids of the type C_{20}, C_{22} and C_{24} as well as C_{16} and C_{18} which however, could not be detected in the mixed fatty acids of the GS_3 from pure butter fat or in the fatty acid of each GS_3 spot from butter fat. The presence of C_{20}–C_{24} acids confirms further the presence of hydrogenated groundnut oil in butter fat. The fatty acids of the GS_3 spot that appeared in the case of adulteration with tallow and Mohua oil below the last spot of butter fat are only composed of C_{16} and C_{18} acids whereas the last GS_3 component of butter fat contains predominantly C_{14} and C_{16} acids and some C_{18} acid.

Thus the presence of the three types of adulterants (studied in the present report) in 5–10% levels can be detected by the separation of GS_3 of butter fat and adulterated butter fat and by subsequent detection of fatty acids. At the same level of adulteration, characteristics such as the refractive index, Reichert-Meissl and Polenske values, saponification and iodine values, did not show any significant deviation from the standard values (see Table II). The observations should prove useful in setting new standards for these fats based on chromatographic separations in addition to providing a simple method for detection of adulteration. It is to be noted that adulteration with tallow and Mohua oil gives the same type of chromatogram of GS_3 as shown in Fig. 1.

Comparison of the chromatogram of the glycerides, as such (Fig. 2), with that of the isolated GS_3 (Fig. 1) of both the pure and adulterated butter fat (5% level) reveals that the separation in the former case is less sharp and the detection of adulteration is more difficult than in the latter case, because of the presence of a large number of mixed saturated–unsaturated glycerides, many of which have the same R_F value and are therefore indistinguishable unless there is a radical change of the fatty acid composition of the glycerides *e.g.* as in hydrogenated groundnut oil. Further work involving quantitative isolation and estimation of GS_3 by argentation TLC is in progress.

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