DETECTION OF REARRANGEMENT REACTION OF NATURAL GLYCERIDES BY CHROMATOGRAPHY

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Rearrangement reactions of glycerides[1-4] have acquired importance in recent years in the preparation of new types of fats by inter- and intramolecular exchange of acyl groups under the influence of catalysts. However, dependable and rapid methods for detecting the change induced have not been developed so far, although detailed[4] analysis may ultimately give the information. It was considered worthwhile therefore to investigate methods for detecting new glycerides formed by this rearrangement reaction.

In recent years chromatographic techniques such as paper and thin layer chromatography are finding enormous applications in the analysis of natural and modified triglycerides. The possibility of applying these in the study of ester rearrangement reactions was therefore investigated. The literature reveals some of the recent studies in this field. Paper chromatography of the more common natural triglycerides has been studied exhaustively by KAUFMANN and co-workers. Improved procedures have been described by KAUFMANN, WESSELS AND VISWANATHAN[5], who separated linseed and sunflower oil glycerides on paper coated with a nonpolar stationary phase like tetradecane and using acetic acid–acetonitrile (8:2) or acetone–methanol (9:1) as mobile phases. STEINER AND BONAR[6] separated cocoa butter glycerides on paper impregnated with 5% liquid paraffin and using acetone–methanol (9:1) as mobile phase.

INOUYE AND NODA[7] separated mixtures of triglycerides by first converting them to mercuric acetate adducts and then fractionating them on paper on the basis of the number of double bonds per glyceride molecule.

Separations and identification of synthetic and natural triglycerides on thin layers of suitable adsorbent have also been studied mainly by KAUFMANN and co-workers[8], who resolved many synthetic triglycerides and the component triglycerides of corn, sunflower, sesame, olive and linseed oils, lard and beef tallow on Kieselguhr plates impregnated with a 5% solution of tetradecane in petroleum ether and using acetone–acetonitrile (8:2) as developing solvent. Using an improved technique, KAUFMANN AND KHOE[9] chromatographed cocoa butter and olive oil on thin layers of calcium sulphate coated with tetradecane. KAUFMANN *et al.*[10] also described the hydrogenation and bromination of the glycerides on plates as steps towards complete separation of the superimposing components. Similar separations of the glycerides of seed oils have been reported by MICHALEC *et al.*[11] on thin layers of silicic acid impregnated with paraffin oil and with acetic acid as developing solvent.

Since the time DE VRIES[12] initially showed that the methyl esters of palmitic,

oleic, and linoleic acids can be separated on the basis of unsaturation on a silicic acid column impregnated with silver nitrate, many investigators have extended this method for elucidation of synthetic and natural, as well as modified, triglycerides on thin layers of silica gel containing silver nitrate. BARRET *et al.*[13] separated and estimated quantitatively triglycerides of lard, interesterified lard, cocoa butter, palm oil, peanut oil, soybean oil, and cotton seed oil on silica gel G plates impregnated with a 12.5 % solution of silver nitrate. Solvents used were 99.5 % chloroform and 0.5 % acetic acid, and a more selective solvent consisting of carbon tetrachloride (60 vol.), chloroform (40 vol.), and acetic acid (0.5 vol), to which variable small amounts of ethanol are added for separation of unsaturated glycerides. After development the plates are charred by spraying with 50 % phosphoric acid and heating to 340°. DE VRIES[14] also separated synthetic glyceride mixtures on silica gel columns impregnated with $AgNO_3$ on the basis of unsaturation and isomeric configurations. REISER *et al.*[15] separated the triglycerides of *Cuphea ilavia var.* miniata seed fat according to the number of double bonds per molecule using preparative thin layer chromatography on silicic acid impregnated with silver ions. The recovered fractions were determined quantitatively by the chromotropic acid technique. The multiple chromatography procedure resolved *Cuphea ilavia* triglycerides into seventeen different components. Chloroform containing 1 % ethanol was used as developing solvent. JURRIENS and co-workers[16] analysed the triglycerides of cocoa butter, Sumatra palm oil, lard, groundnut oil, soybean and cotton seed oils according to their degree of unsaturation by means of thin layer chromatography on silica gel G impregnated with $AgNO_3$. The glycerides are extracted from the adsorbent and the amount in each fraction is determined by glycerol determination with periodic acid after saponification. PRIVETT *et al.*[17] separated a randomised mixture of synthetic triglycerides containing palmitic, oleic and linoleic as well as palmitic, oleic and linolenic on silicic acid impregnated with silver nitrate as described by BARRETT *et al*[13]. The less unsaturated triglycerides (3 or less double bonds) are chromatographed with 0.8 % methanol in chloroform. Triglycerides containing more than three double bonds are chromatographed with 2–3 % methanol in chloroform.

KAUFMANN AND WESSELS[18] separated the glycerides of sunflower oil by first fractionating on silver nitrate impregnated silica gel plate and effecting further separation in a reversed phase system.

In the present study, thin layer and paper chromatography techniques have been used to separate the glycerides of some seed oils, before and after rearrangement reactions, by the reversed phase multiple development principle. The object of such separations has been to note their pattern and the nature of the new component glycerides produced as a result of random rearrangement.

EXPERIMENTAL

Paper chromatography

Whatman No. 1 chromatography paper was used for the separation of the glycerides. The paper strips (14 in. × 3 in.) were impregnated with a 5 % solution of liquid paraffin (B.P., B.D.H.) in petroleum ether (b.p. 40–60°). 100 μg of the original and randomised samples were then spotted on the impregnated papers and three developments were carried out in the ascending manner using acetone–methanol

TABLE I

PAPER CHROMATOGRAPHY OF NATURAL AND RANDOMISED GLYCERIDES

Glyceride sample	*Number of spots*	*R_F value of spot* (× 100)*											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
Natural groundnut	6	10.4	16.8	24.1	31.8	39.1	90.0						
Randomised groundnut	9	7.7	14.1	20.0	26.3	33.2	40.4	87.7	92.7	97.2			
Natural sesame	6	16.8	23.6	30.0	33.2	45.4	92.7						
Randomised sesame	8	16.0	23.6	30.4	33.2	45.0	84.0	91.3	97.3				
Natural cottonseed	5	34.0	41.6	50.4	60.0	90.0							
Randomised cottonseed	6	34.0	42.4	51.2	60.0	88.0	92.0						
Natural safflower	6	37.6	45.0	53.6	61.6	77.3	90.8						
Randomised safflower	7	37.0	46.0	54.4	62.4	69.2	88.8	93.2					
Natural linseed	9	38.0	45.6	53.2	61.2	68.8	76.0	80.8	89.2	93.6			
Randomised linseed	10	38.4	45.2	54.4	62.0	69.2	76.0	82.0	86.8	91.6	96.8		
Natural mustard	6	14.4	20.4	26.8	32.0	38.0	46.0						
Randomised mustard	12	10.0	15.6	20.4	26.0	31.6	38.4	46.0	52.8	74.8	78.4	83.2	88.4

* Spots serially numbered from the base line.

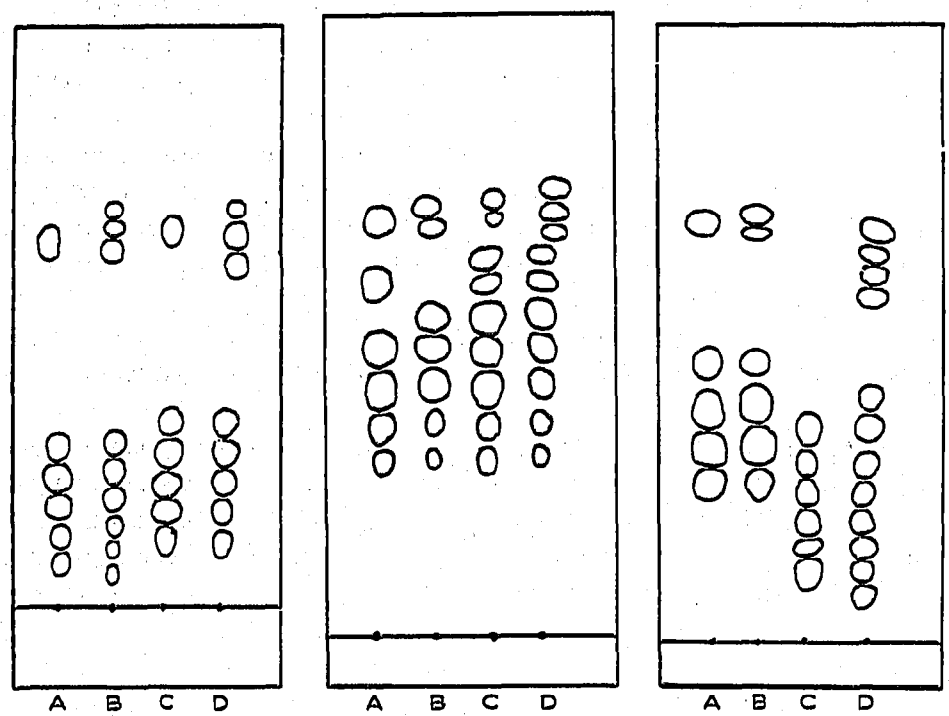

Fig. 1. Chromatographic separation of: (A) Natural groundnut glycerides; (B) Randomised groundnut glycerides; (C) Natural sesame glycerides; (D) Randomised sesame glycerides.

Fig. 2. Chromatographic separation of: (A) Natural safflower glycerides; (B) Randomised safflower glycerides; (C) Natural linseed glycerides; (D) Randomised linseed glycerides.

Fig. 3. Chromatographic separation of: (A) Natural cotton seed glycerides; (B) Randomised cotton seed glycerides; (C) Natural mustard glycerides; (D) Randomised mustard glycerides.

(8:2) as eluting solvent. After development, the papers were freed from solvent and exposed to iodine vapour for 30 min, and the back was sprayed with 1% starch solution[6] when white spots on a blue background were observed.

The number of spots and their R_F values are given in Table I.

The separations are shown in Figs. 1, 2 and 3.

Thin layer chromatography

The separation of triglycerides was carried out on Kieselguhr plates impregnated with liquid paraffin. The plates were prepared by pouring a slurry of 6 g Kieselguhr (E. Merck), 0.6 g plaster of Paris (E. Merck) and 13.2 ml of distilled water in an applicator designed in the laboratory and adjusted to a clearance of about 0.3 mm and drawing the applicator along the glass plates (20 × 10 cm). The plates were allowed to set for 15 min, then baked at 110° for 1 h and finally stored over fused calcium chloride in a desiccator.

The plates were impregnated with 5% solution of liquid paraffin (B.P., B.D.H.) in petroleum ether (b.p. 40–60°) and left in the air for 10 min to remove petroleum ether. After spotting exactly similar amounts of the original and randomised samples (as a 1% solution in benzene; E. Merck) on the impregnated plates, the plates were developed in closed chambers with 8:2 and 7:4 acetone–methanol, previously saturated with liquid paraffin. The glycerides of groundnut, sesame and mustard oils were separated by acetone–methanol (8:2) and acetone–methanol (7:4) was employed for safflower, cotton seed and linseed oil glycerides. The plates were developed twice except in the case of the mustard oil plate which was developed four times. The plates, after development, were freed from solvent and exposed to iodine vapour for about 5 min. When brown spots appeared the plates were taken out and sprayed with 1% α-cyclodextrin in 30% ethanol when blue spots appeared against a white background.

The number of spots and their R_F values are given in Table II. Separations are shown in Figs. 4, 5, 6 and 7.

DISCUSSION

In the present investigation seed oils containing high percentages of unsaturated acids have been randomly rearranged. The component glycerides of these oils usually have a high mobility. After the rearrangement some of the component glycerides were found to separate with even higher mobility. These higher mobility components are obviously more unsaturated glycerides and are composed, wholly or predominantly, of C_{18} unsaturated acids. There were, however, two extra spots at the bottom of the TLC plate and one on the paper in the case of mustard seed oils which are likely to contain erucic acid or other higher saturated acids (C_{20} and above), and the mobility of the glycerides of these acid radicals is quite low. The number of spots separated may represent either individual glycerides or critical partners. There was a difference between the original and rearranged seed oils in the number of spots and the rearranged oils showed an increase in the number of component glycerides. In fact, rearrangement in general increases the number of component glycerides. Although the number of spots obtained by thin layer and paper chromatography differ, the separations achieved demonstrate that these two techniques can be readily used for rapid detection of rearrangement reactions. It is also obvious that a rearrangement

TABLE II

THIN LAYER CHROMATOGRAPHY OF NATURAL AND RANDOMISED GLYCERIDES

Glyceride sample	*No. of spots*	*R_F value of spot* (× 100)*									
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Natural groundnut	8	29.7	36.2	42.4	50.7	59.4	68.1	75.3	81.1	—	—
Randomised groundnut	10	29.0	34.7	41.3	48.5	58.0	66.6	73.1	79.7	85.5	93.4
Natural sesame	5	39.1	47.1	55.8	63.7	71.7					
Randomised sesame	7	40.6	48.5	57.2	65.2	73.9	86.9	94.2			
Natural cotton seed	4	52.9	61.6	70.3	78.2						
Randomised cottonseed	5	52.2	61.6	70.3	77.5	96.4					
Natural safflower	4	55.8	63.0	71.1	78.2						
Randomised safflower	6	55.8	63.0	71.1	78.2	84.0	96.4				
Natural linseed	7	50.7	55.7	62.8	69.2	74.2	80.0	82.9			
Randomised linseed	8	51.4	57.7	65.5	71.1	76.0	80.0	84.5	97.1		
Natural mustard	6	57.2	61.7	69.1	76.6	84.0	89.5				
Randomised mustard	9	40.9	47.6	55.0	62.1	69.1	76.6	84.0	89.2	94.4	

* Spots serially numbered from the baseline.

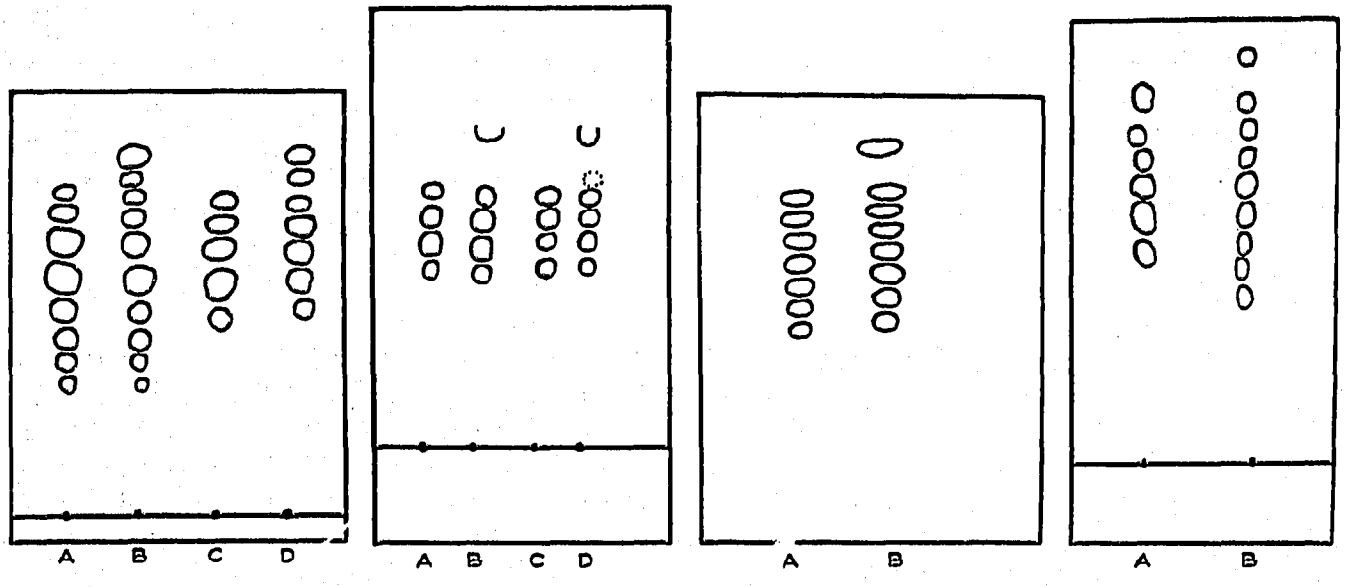

Fig. 4. Chromatographic separation of: (A) Natural groundnut glycerides; (B) Randomised groundnut glycerides; (C) Natural sesame glycerides; (D) Randomised sesame glycerides.

Fig. 5. Chromatographic separation of: (A) Natural cotton seed glycerides; (B) Randomised cotton seed glycerides; (C) Natural safflower glycerides; (D) Randomised safflower glycerides.

Fig. 6. Chromatographic separation of: (A) Natural linseed glycerides; (B) Randomised linseed glycerides.

Fig. 7. Chromatographic separation of: (A) Natural mustard glycerides; (B) Randomised mustard glycerides.

reaction which alters the glyceride composition and results in an increase of the number of spots compared with the original oil can also be used for the detection of adulteration of fats, as adulteration with any other oil would lead to a change in the number and intensity of the spots.

Further work for identification of new components of glycerides formed by the rearrangement reaction is in progress.

SUMMARY

The techniques of reverse phase paper and thin layer chromatography have been applied for the rapid detection of the rearrangement reaction involving randomisation of some seed oils containing high percentages of unsaturated acids. It has been found that the two techniques separate the component glycerides readily permitting visualization and detection of the effect of such rearrangements on glyceride composition.

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