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Chromatographic investigation of the component glycerides of kusum oil

Kusum or macassar oil is the fat available from the seeds of *Schleichera trijuga* belonging to the family *Sapindaceae*. The kusum tree is almost wholly indigenous to India, growing widely in the sub-Himalayan region, throughout central and southern India, Burma, Ceylon, Java and Timor. A notable feature of the oil is the characteristic presence of a cyanogenic compound to the extent of 0.03–0.05% as HCN[1]. This cyano compound has recently been identified by KUNDU *et al.*[2] as being a cyano glyceride. Very little work appears to have been done on the component glycerides of the oil. The only notable work in this respect is that of DHINGRA *et al.*[3], who oxidised the fat with permanganate in acetone solution and analysed the products with respect to acid value, saponification value and iodine value.

In the present investigation adsorption, argentation and reversed phase thin layer chromatography (TLC) have been applied to study the component glycerides of the oil. The results of this chromatographic approach are now presented.

Separation of the triglyceride components by adsorption chromatography

Of 5% solution of the crude oil in chloroform 40 mg was applied as a band on three plates of Silica Gel G (0.8 mm) and fractionated, using the solvent system *n*-hexane–diethyl ether–acetic acid[4] in the ratio 75:25:1. Suitable standards consisting of a triglyceride (tripalmitin), a fatty acid and a sterol were run side by side for comparison. The bands were located by exposure to iodine vapour. The triglyceride bands were marked, scraped off the plates into a 100 ml erlenmeyer flask after removal of adsorbed iodine, extracted with warm chloroform and filtered. The filtrate was then concentrated to a *ca.* 1% solution by evaporation of the solvent under reduced pressure.

Subfractionation of the triglyceride components by argentation TLC

The solution of the triglyceride components was applied as a band on three plates of Silica Gel G (0.5 mm) impregnated with silver nitrate and then developed, using the solvent system chloroform–acetic acid (100:0.5)[5]. The bands were visualised by spraying with a 0.2% solution of 2′,7′-dichlorofluorescein in 95% ethanol, followed by exposure under U.V. light. The chromatogram is presented in Fig. 1. After subfractionation, the individual glycerides having identical band positions were combined, extracted with dry diethyl ether and freed from the dye according to the procedure of KAUFMANN AND WESSELS.[6]

Liberation of the fatty acids from the individual glycerides

The solvent was removed by evaporation from each fraction. Each of the fractions was next saponified with 1 *N* ethanolic potassium hydroxide, freed of ethanol under reduced pressure, acidified to liberate the fatty acids, extracted with ether and washed free of mineral acid with water. The ether was finally removed and the residual fractions were each taken up in 0.5 ml chloroform.

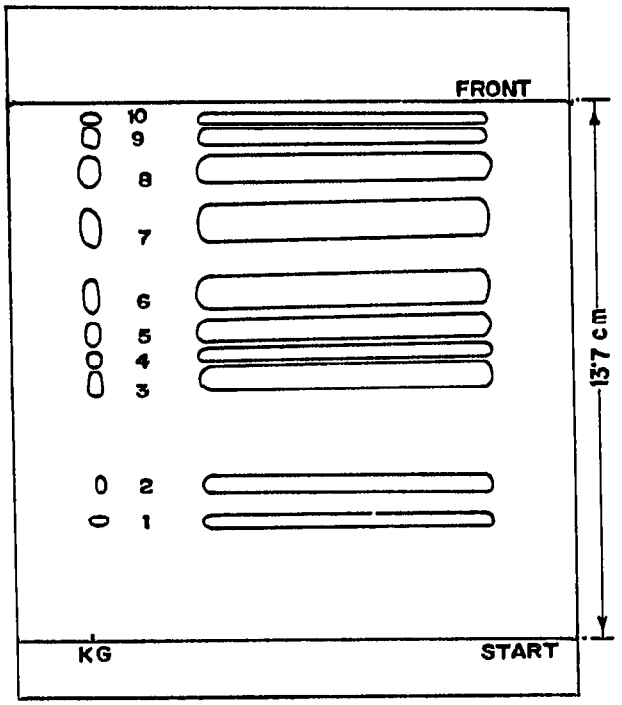

Fig. 1. Thin layer chromatogram of kusum glycerides (KG) on Silica Gel G layer impregnated with silver nitrate. Developing solvent: chloroform–acetic acid (100:0.5). Bands were located by spraying with 0.2% solution of 2′,7′-dichlorofluorescein in 95% ethanol, followed by exposure under U.V. light and were reproduced by tracing. For explanation of numbers see Table I.

Fractionation of the fatty acid components in the individual glycerides by reversed phase TLC

The fatty acids of each glyceride fraction were fractionated on a plaster of Paris plate coated with a 5% solution of liquid paraffin in petroleum ether[7] and identified by comparison with standard fatty acids run side by side under identical

TABLE I

FATTY ACID COMPOSITION[a] OF THE GLYCERIDE FRACTIONS OBTAINED BY THIN LAYER CHROMATOGRAPHY ON SILVER NITRATE IMPREGNATED SILICA GEL PLATES

Fatty acids	*Component glycerides numbered serially from base line upwards*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Capric	—	+	—	—	—	—	—	—	+	—
Lauric	—	—	—	—	—	—	—	—	—	—
Myristic (Linoleic)	—	—	—	—	—	+++	—	—	—	—
Palmitic (Oleic)	+++	+++	++	+++	+++	++	++	++	++	+
Stearic	+	+	—	+	++	—	+	++	—	+
Arachidic	—	—	+	—	—	—	—	++	+++	++
Behenic	—	—	+	—	—	—	—	+	—	—

[a] + sign indicates the approximate concentration of the component fatty acids present in the glyceride fractions. An increased number of + signs implies increased concentration.

conditions. The solvent systems used for development were: (i) 90% acetic acid for higher molecular weight fatty acids, and (ii) 70% acetic acid for relatively lower molecular weight acids. The spots were visualised by spraying with 1% solution of α-cyclodextrin in 30% ethanol and exposure to iodine vapour. The results are recorded in Table I.

The presence of oleic acid in the glyceride fractions 1–3, 8 and 9 (Fig. 1) was indicated by conversion to the bromo derivative and subsequent resolution according to the two-dimensional chromatographic procedure of KAUFMANN *et al.*[8].

Discussion

Subfractionation of the triglyceride components according to their degree of unsaturation and isomeric configuration by argentation TLC yielded ten spots. Each of these spots may represent a single glyceride or critical partner(s) having identical R_F values. Further, the presence of a single fatty acid alone has not been indicated in any one component glyceride. It may be possible that this is again due to the presence of some critical partner(s). In any event, the observations combined together indicate in general that the structure of the kusum glycerides conforms to a pattern of more or less even distribution of the fatty acids among the glycerol molecules. This general structure of the oil is consistent with that of other vegetable kernel fats and essentially substantiates the conclusion made by DHINGRA *et al.*[3].

Table I indicates the fatty acid compositions of the component glycerides numbered serially from the baseline upwards in the chromatogram (Fig. 1). Two-dimensional chromatographic analysis of the glyceride fractions 1–3 and 8 and 9 reveals that the predominant constituents of fractions 1–3 are oleic acid, while bands 8 and 9 are composed of predominantly saturated acids. It has been further observed that cyanoglycerides are present in bands 8 and 9 (ref. 2). Presence of cyano compounds implies increased polarity of these fractions. Consequently, the fractions would be expected to be in the lower half of the chromatogram. This seeming contradiction with the normal chromatographic behaviour may be attributed to the reduced molecular weight[2] of the cyanoglycerides and to their predominantly saturated fatty acid constituents.

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Department of Applied Chemistry,
Calcutta University,
Calcutta-9 (India)

M. K. KUNDU

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