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[Received September 7, 1979]

## ❖ Chemical Investigation of the Seeds of *Brassica oleracea* Var. *Acephala*

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### ABSTRACT

Fatty acid composition of the seed fat of *Brassica oleracea* Var. *acephala* (Cruciferae) has been determined. Erucic acid has been found to be the major component followed by linoleic, oleic, linolenic, arachidic and palmitic acids. Traces of stearic and eicosenoic acids have also been detected. The unsaponifiable matter contained  $\beta$ -sitosterol, and defatted seeds showed the presence of sucrose.

### INTRODUCTION

*Brassica oleracea* Var. *acephala* — commonly known as "Kale" (1) — is a tall pot herb with curled leaves, grown in Assam, Bombay, Baroda, and Kashmir. The fatty acid composition of its seeds has not been worked out so far. Earlier detailed analyses of some allied species have been made (2), and the occurrence of eicosenoic acid in the seed fat has been confirmed (3).

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### EXPERIMENTAL

About 300 g of the seeds were partially ground and extracted for 16 hr with petroleum ether (40-60 C) in a soxhlet. Removal of the solvent gave a yellow fixed oil in 6.5% yield. The physical constants of the oil were determined (Table I). It was saponified, and the mixed acids so

TABLE I

Physicochemical Properties of the Oil

| Determination            | Value  |
|--------------------------|--------|
| Specific gravity at 20 C | 0.9010 |
| Refractive index at 23 C | 1.4741 |
| Optical rotation at 23 C | -0.4'  |
| Acid value               | 2.1    |
| Iodine value             | 61.2   |
| Saponification value     | 123.06 |
| Acetyl value             | 89.8   |
| Unsaponifiable matter    | 1.6%   |

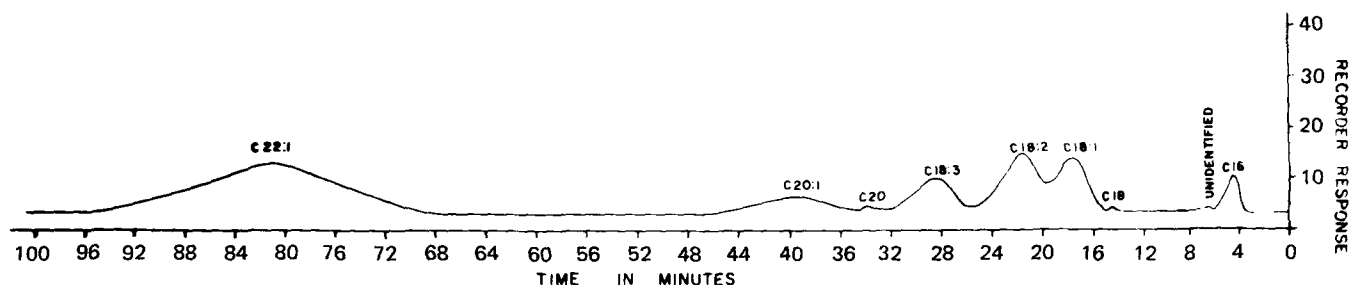


FIG. 1. Gas liquid chromatogram of the methyl esters of mixed acids on Reoplex column.

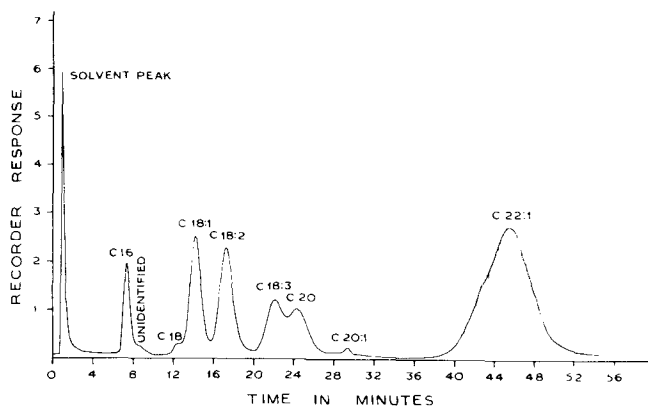


FIG. 2. Gas liquid chromatogram of the methyl esters of mixed acids on DEGS column.

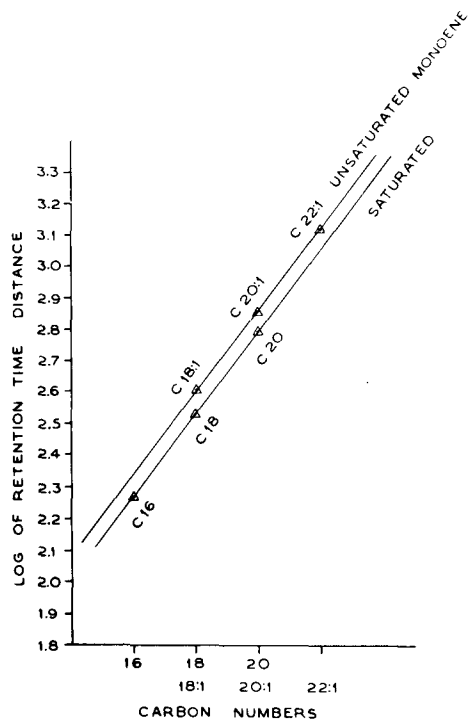


FIG. 3. Log plot of retention time distance of saturated and unsaturated methyl esters against carbon numbers.

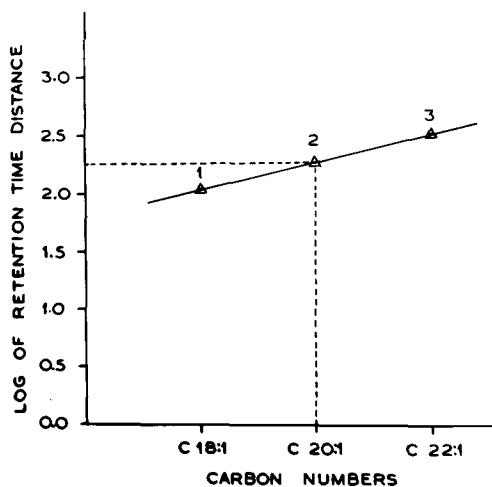


FIG. 4. Log plot of retention time distance of unsaturated methyl esters against carbon numbers.

obtained were further resolved into saturated and unsaturated ones by the lead salt method.

The mixed fatty acids were converted into their methyl esters and then analyzed by GLC (Perkin Elmer 881) using Reoplex (15% supported on Chromosorb KV in a stainless steel column of 3 ft length and 1/4" diameter, at a temperature of 180 C, using flame ionization detector, nitrogen as carrier gas and a chart speed of 12 mm/min) and DEGS (20% supported on chromosorb W in a stainless steel column of 6 ft length and 1/4" diameter, at a temperature of 185 C, using flame ionization detector, nitrogen as carrier

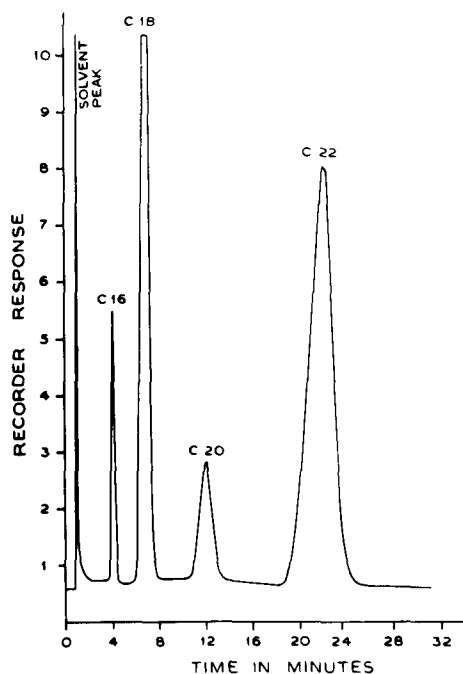


FIG. 5. Gas liquid column chromatogram of the methyl esters of hydrogenated product on Silicone (SE-30) column.

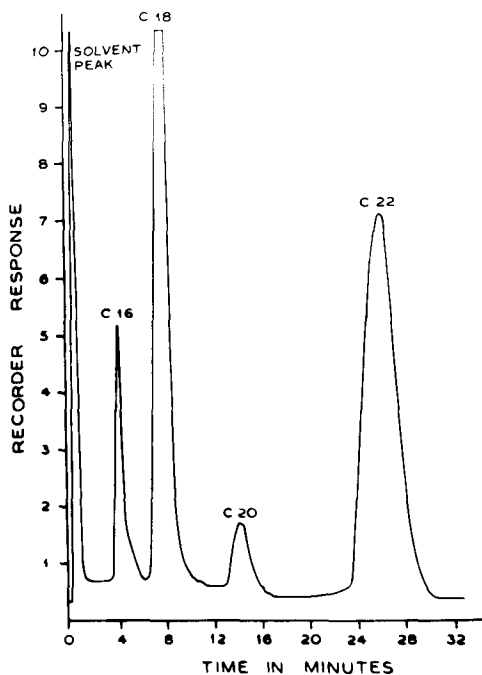


FIG. 6. Gas liquid chromatogram of the methyl esters of hydrogenated product on polyester (Reoplex) column.

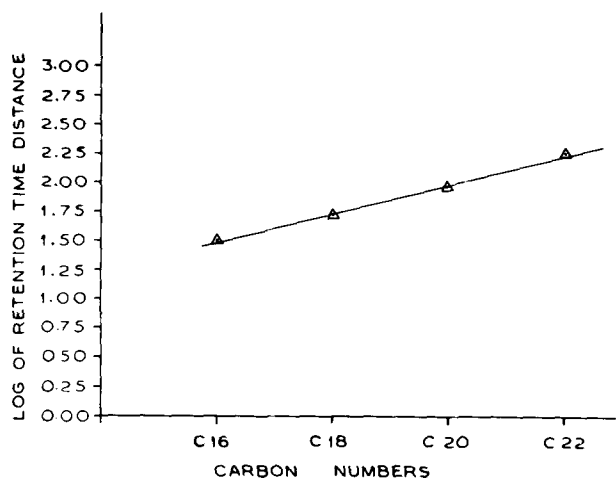


FIG. 7. Log plot of retention time distance of hydrogenated methyl esters against carbon numbers.

gas and a chart speed of 12 mm/min) columns. Two types of columns were used since Reoplex column did not give a clear separate peak for eicosenoic acid, which overlapped that of arachidic acid.

## RESULTS AND DISCUSSION

The ethereal solution of unsaponifiable matter was dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure gave a yellowish solid mass (2 g), which was crystallized with petroleum ether (40-60 C). The crystalline matter responded to the Libermann-Burchard test, was found to have mp 135-36 C, and Rf value 0.5 (TLC using petroleum ether/ethyl acetate 75:25). The compound was acetylated (pyridine/acetic anhydride),

and mp of acetate was found to be 124-26 C with Rf value 0.8 (TLC using benzene).

The defatted seeds, left after petroleum ether extraction, were subjected to successive solvent extraction starting from nonpolar to polar solvents. The acetone extract (left overnight) gave white shining crystals (2 g). The crystals responded to the Molish test but did not reduce the Fehling solution. The compound was hydrolyzed by HCl and neutralized with NaOH. It was found to have Rf value 0.23 (paper chromatography using n-butanol/acetic acid/water, 4:1:5).

Table I shows the properties of the seed oil. The fatty acid composition (in terms of esters) was 4.4% palmitic, 0.7% stearic, 11.3% oleic, 12.6% linoleic, 10.2% linolenic, 8.2% arachidic, 0.4% eicosenoic and 51.8% erucic. The unsaponifiable matter has been identified as  $\beta$ -sitosterol, and the defatted seeds have been found to contain sucrose.

## ACKNOWLEDGMENT

The help of Dr. K.L. Bedi and Miss R.K. Jamwal of RRL Jammu, Dr. A.K. Kalla of Kashmir University and Dr. S.D. Bhagat of RRL Jorhat is acknowledged. Thanks are due to the UGC for Research Fellowships to V.K. Kaul and A. Banerjee.

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[Received May 1, 1979]