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# THE SEPARATION OF SATURATED AND UNSATURATED FATTY ACID ESTERS BY GAS-LIQUID CHROMATOGRAPHY<sup>1</sup>

B. M. CRAIG AND N. L. MURTY<sup>2</sup>

## ABSTRACT

Fatty acid methyl esters are separated according to chain length by using washed silicone grease supported on 20-40 mesh C<sub>22</sub> firebrick. By using a plasticizer on the firebrick support the same esters are separated according to both chain length and degree of unsaturation. Together the two procedures can be used to determine the component fatty acids in fats and oils. Nearly parallel linear relations are obtained for saturated, monoethenoid, diethenoid, and triethenoid methyl esters when the logarithm of emergence time is plotted against the carbon number of the fatty acid.

## INTRODUCTION

The development of gas-liquid partition chromatography by Martin and James (1, 2) has provided a new and powerful tool for semimicro- and micro-analyses. The rapid extension of this method to fats and oils by Martin and James (1, 2, 3), Cropper and Heywood (4, 5), Dijkstra *et al.* (6), Van de Kamer *et al.* (7), Ray (8) and the improvements developed by these workers have made possible the separation of small quantities of fatty acid esters. These separations are based on chain length and include separation of esters of odd-numbered acids and branched-chain acids. Silicone grease and Apiezon grease with or without stearic or other saturated acids have been the preferred liquid phases supported on Celite or crushed firebrick. A further development has been fractionation on the basis of unsaturation in a series of esters such as stearic, oleic, linoleic, and linolenic acids. This has been accomplished by Orr and Callen (9) and by Lipsky and co-workers (10) using commercial plasticizers as liquid phases. The first used by Orr and Callen (9) is Reoplex 400, which is a commercial plasticizer and is a polyoxyalkalene adipate. The plasticizers used by Lipsky are adipic esters of diethylene glycol and the adipic ester of diethylene glycol cross-linked with pentaerythritol.

In this laboratory gas-liquid chromatography has been used with silicone and Apiezon liquid phases for the analysis of fats and oils. Reoplex 400 was found to "bleed" extensively, particularly at temperatures over 200° C. Attempts were then made to synthesize plasticizers which would effect the separation of stearic, oleic, linoleic, and linolenic acids but which would not "bleed". A number of these have been synthesized and tested and the results are given in this paper.

## EXPERIMENTAL

### *Silicone Column*

Dow-Corning High Vacuum stopcock grease was dispersed in ethyl acetate by means of an Ultra-Turrax high speed stirrer, then precipitated out by addition of methyl alcohol and treated as described by Cropper and Heywood (4, 5). The washed silicone grease was dispersed in ethyl acetate in a round-bottom flask. C<sub>22</sub> firebrick (Johns Manville) was added to the suspension (a mixture of 20-30 and 30-40 mesh 1:1 (w/w)), and the solvent evaporated at 80-90° C under vacuum in a laboratory rotary evaporator.

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<sup>2</sup>National Research Council of Canada Postdoctorate Fellow, 1957-.

The silicone grease was applied in a 1:4 ratio (w/w) with the crushed firebrick, and was packed in a copper column  $\frac{1}{4}$  inch in diameter by 12 feet in length.

#### *Plasticizer Columns*

Two plasticizers were synthesized using a dibasic acid and diethylene glycol containing 1% by weight of diglycerol to provide cross-linking. The dibasic acids were adipic and succinic and details are given for preparation of the succinic plasticizer. A mixture of 24.0 g succinic acid, 21.2 g of diethylene glycol, 0.5 g of diglycerol, and 0.2 g of *p*-toluenesulphonic acid in a round-bottom flask was heated at 130° C/2 mm for 2 hours with occasional shaking. A clear sirupy solution resulted. The plasticizer was dissolved in ethyl acetate, washed three times with water, dried over anhydrous sodium sulphate, filtered, and the solvent removed under reduced pressure. The plasticizer was dissolved in ethyl acetate and applied to 20–40 mesh C<sub>22</sub> firebrick in a 1:4 ratio (w/w) as outlined for the silicone column.

Two columns, copper tubing  $\frac{1}{4}$  inch by 6 feet long, using succinic and adipic plasticizers, respectively, were coupled in series, with the succinic as the fore column. Initial tests indicated that the succinic plasticizer gave a more effective separation of the esters of stearic and oleic acids and the adipic column of the esters of linolenic and arachidic acids.

#### *Apparatus*

The gas-liquid chromatographic unit of conventional design was constructed in the laboratory. Four Gow-Mac filaments were employed with two in each of the reference and sample detectors and the bridge was operated at 150 ma. Helium was used as the carrier gas and the recorder was operated at the 2.5-mv range. The apparatus was controlled at a temperature of 210° C. The silicone column was operated at a flow rate of 100 cc per minute and the plasticizer columns at 70 cc per minute, as measured by a soap bubble unit on the outlet tube. The sample sizes varied from 3 to 15  $\mu$ l, depending on the particular oil, and injection was made with a precision syringe.

The materials used in the study were the methyl esters of the fatty acids from tallow, soybean oil, lard, and herring oil, which were kindly supplied by the research laboratory of Canada Packers Limited, Toronto, Canada.

### RESULTS AND DISCUSSION

Figure 1, which reproduces actual recorder charts, gives the curves that resulted for lard, tallow, soybean oil, and herring oil esters with the plasticizer column and for lard and herring oil esters with the silicone column. The position of peaks representing C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> saturated acids, labelled 1, 2, 3, 4, and 5, respectively, were determined by calibration with a known synthetic mixture of these acids.

The separation of palmitic, stearic, oleic, linoleic, and linolenic methyl esters in soybean oil are comparable to those obtained by Lipsky (10) using a commercial plasticizer. The plasticizer used in the present work "bled" slightly during the first runs but little or not at all at later stages and operation of the column for a period of 3 weeks indicated no decreased efficiency. The present columns using a 1:4 ratio of liquid phase have a limited capacity, e.g. approximately 10 mg of soybean esters on 20 g of column packing before evidence of overloading is indicated.

The tallow esters on a silicone column showed C<sub>8</sub>, C<sub>10</sub>, C<sub>12</sub>, C<sub>14</sub>, C<sub>15</sub>, C<sub>16</sub>, C<sub>17</sub>, and C<sub>18</sub> acids as well as peaks between C<sub>14</sub> and C<sub>15</sub> and C<sub>16</sub> and C<sub>17</sub>, which may have been branched-chain acids. The tallow esters from the plasticizer column showed additional peaks which may be due to unsaturated acids of various chain lengths. Herring oil esters

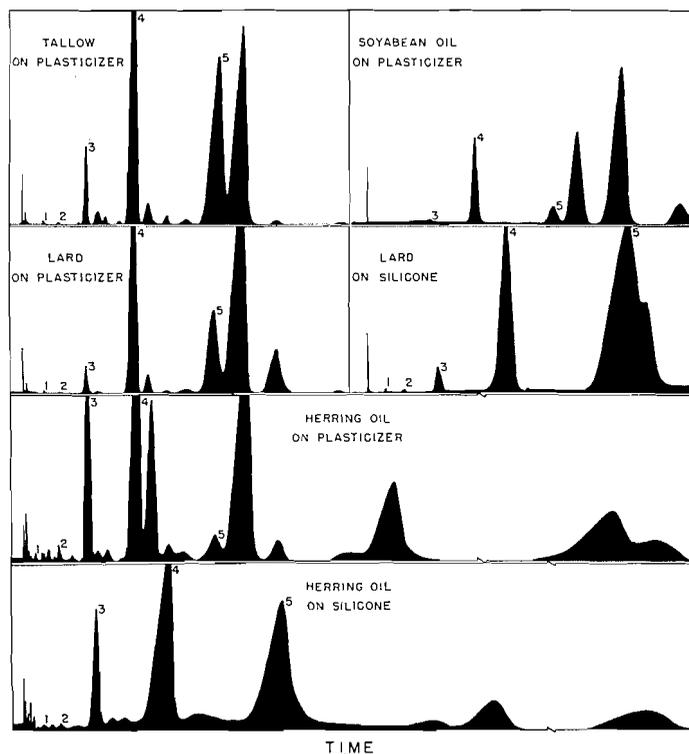


FIG. 1. Gas-liquid chromatographic separations of methyl esters of fats and oils on silicone and plasticizer columns. The numbers 1, 2, 3, 4, and 5 refer to the saturated  $C_{10}$ ,  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$  acids. Peaks following these are odd-numbered or branched-chain in the case of silicone columns or unsaturated from the plasticizer column.

also showed odd-numbered fatty acids,  $C_9$ ,  $C_{11}$ ,  $C_{13}$ , and  $C_{15}$ , in addition to the expected even-numbered series on a silicone column. The lard esters showed even-numbered members from  $C_{10}$  to  $C_{20}$  on a silicone column and a number of unsaturated acids in each group on the plasticizer column.

Some separation of saturated and unsaturated acids took place on the silicone column within a group such as the  $C_{18}$  acids. The separation was the reverse of that accomplished by the plasticizer column in that the less unsaturated acids are retained more strongly by the liquid phase. This was proved by coupling a silicone column to a plasticizer column. The separation on the plasticizer then offset the silicone column, single peaks being obtained for each chain length. The same results were obtained with columns using Apiezon "M" as a liquid phase coupled to a plasticizer column.

The logarithm of the emergence time (time from the air peak to the sample peak) has been plotted against number of carbon atoms (Fig. 2). Peaks representing the saturated acids  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$  were plotted first and the linear relation for chain length against logarithm of retention time was established. Other peaks from various curves were plotted on the same line according to retention time only. The odd-numbered acids and compounds thought to be branched-chain acids are also represented, both from the silicone and the plasticizer columns.

The "monoethenoid" relation was made up by moving the points representing the peaks following those of palmitic, stearic, and arachidic acids, e.g. palmitoleic, oleic, and

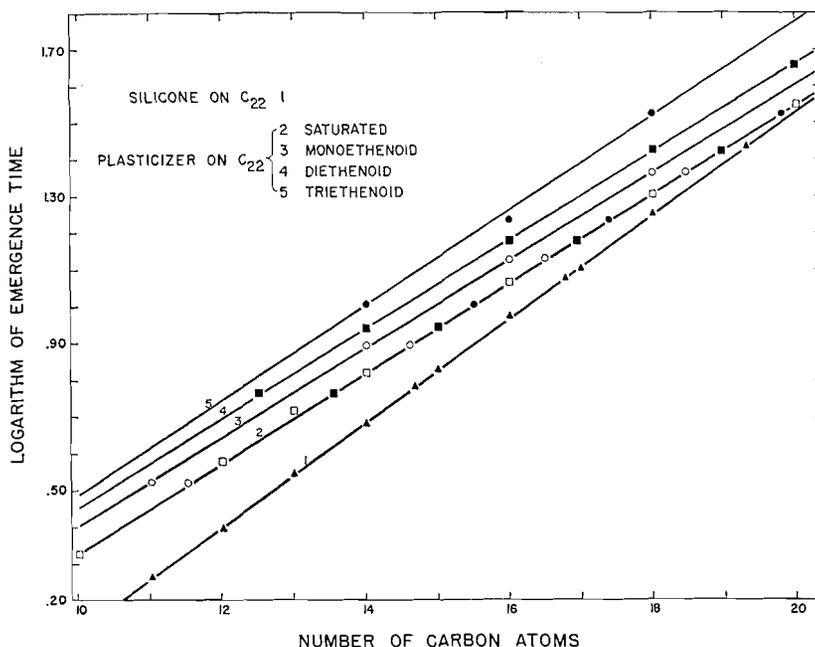


FIG. 2. Logarithm of emergence time plotted against carbon number for saturated, monoethenoid, diethenoid, and triethenoid acids from plasticizer columns and for acids from silicone column.

eicosenoic acids, to correspond to the appropriate carbon numbers. As expected, a linear relation was obtained, since the presence of one double bond would presumably have the same influence on each chain length.

The same procedure was followed for the diethenoid acids, giving a family of linear relations which are essentially parallel. The linear relation for triethenoid does not seem to be parallel to the other relations but further work needs to be done on polyethenoid acids with polyester columns to establish the slope of this line in relation to the less unsaturated acids.

Since these linear relations were established by using known esters and fatty acid esters of oils of known composition, the system is applicable for analysis of any other oil if similar conditions of temperature, flow rate, column size, and packing are used. The present system of plasticizers alone is inadequate, since the dienoic  $C_{16}$  acid has the same emergence peak as the  $C_{17}$  acid. Similarly, linolenic acid and arachidic acid examined in other oils show a simultaneous emergence. Such difficulties could be resolved by collecting the fractions from the silicone column and applying these in turn to a plasticizer column, a procedure which would require the initial separation of larger quantities of fatty acid esters.

The research on separation of saturated and unsaturated fatty acids is being continued to find liquid phases which give a more efficient separation particularly of such sequences as stearic-oleic and linolenic-arachidic acids. Preliminary results on quantitative measurements of unsaturated acids such as linoleic and linolenic which are presently done by spectrophotometric analysis indicate that the chromatographic method is equally reliable and requires considerably smaller samples.

## ACKNOWLEDGMENTS

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