Gas-Liquid Chromatographic Determination of the Fatty Acid Composition in Oil from Seeds of *Ulmus*

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By means of low-pressure chromatography methyl esters of the fatty acids in seeds from various species and varieties of the genus *Ulmus* have been determined. The statements in the literature according to which elm seed oil has a surprisingly high content of C₁₀ fatty acids has been confirmed.

Gas-liquid chromatography was introduced in 1952 by James and Martin¹ and used for instance for the separation of volatile fatty acids with up to 11 carbon atoms on a column with Celite impregnated with silicon fluid in which 10 % stearic acid has been dissolved and using nitrogen as carrier gas. Later James and Martin² extended the applicability of the method to fatty acids with up to 18 carbon atoms, separating the methyl esters on a Celite column impregnated with Apiezon vacuum stopcock grease at 197°C. Cropper and Heywood³ state that they are able to separate fatty acids with up to 22 carbon atoms as methyl esters at 230°C and a pressure of 20 mm Hg at the column outlet. Their column was packed with Celite impregnated with Dow Corning silicone grease and the carrier gas used was nitrogen. However, the pressure drop along a Celite column is great, and Cropper and Heywood⁴ have therefore proposed the use of sodium chloride crystals to obtain a reduction of the pressure drop to one twentieth of that of the Celite column.

Since February 1955 work on gas-liquid chromatography has been in progress in »Laboratoriet for almen teknisk Kemi» with a view to the development of methods for quantitative analysis of mixtures of methyl esters of fatty acids. In the present article an account will be given of the experience gained with regard to suitable equipment, and the method is illustrated through an examination of oil from seeds of the genus *Ulmus*.

EQUIPMENT

Column. The chromatographic column (Fig. 1) is made up of four stainless steel tubes, each 1 meter long and secured by brazing to a top and a bottom flange. The bore of the tubes is 8 mm. The bottom flange is closed by means of a plate and a lead gasket by

Fig. 1. Chromatographic column. a. Nitrogen inlet tube, in which the ampoule breaker is placed. b. Column tube. c. Furnace tube for heating of the column. d. Catharometer wire support. e. Catharometer block. f. Cold trap. g. Reheating tube. h. Nitrogen outlet.

Fig. 2. Sensitive element of the catharometer. a. Platinum filament. b. Platinum wire. c. Glass support.

means of which it is possible to connect all four tubes in series to form one column (4 m long) or to connect two tubes only to form a 2 m column. The detector is secured to the top flange by means of bolts and a lead gasket.

The detector. The detector used is a catharometer prepared from a brass block with four borings lined with stainless steel tubes of the same type as those used for the column. Two of the tubes (d) in the block are used for measurements of the thermal conductivity of the gas flow from the column, both directly and after removal by condensation of entrained material in a U-tube cooled in a mixture of solid carbon dioxide and acetone. In Fig. 1 this cooling-tube is shown attached to the column. The third tube (g) in the catharometer block is used for reheating the gas flow after the cooling process. The thermal conductivity is measured by means of two electrically heated platinum filaments which form a Wheatstone bridge together with two permanent resistances. The bridge is supplied with current from a storage battery which is charged continuously during the experiment. A 5 mV recording potentiometer is connected across the Wheatstone bridge. The platinum filaments are finely coiled and attached to platinum wires sealed into a glass support (Fig. 2). The sensitivity of the catharometer has been found by calculation to be $5/16 \mu\text{g/ml N}_2$ at 20 mm Hg and 8 ml N_2/minute both for C_8 and C_{12} methyl esters.

Ampoule breaker. The fourth tube (a) in the catharometer block is used for the admission of nitrogen to the column. It also contains an ampoule breaker in which the small spherical glass ampoule with the weighed sample of methyl esters is placed at the beginning of the run. When the appropriate temperature and nitrogen flow conditions are attained the ampoule is broken admitting the sample to the column without interruption of the nitrogen stream.

The column with its detector is surrounded by an electrically heated furnace tube, in which the temperature is kept constant by means of a regulating transformer.

The pressure at the column outlet is kept constant by means of a manostat consisting of a 1 litre reference flask placed in a water bath and connected to one side of a regulating (electric contact) pressure gauge, the other side of which is connected to the system. The pressure gauge, which contains diethylene glycol, controls a magnetic air inlet valve through an electronic relay.

The rate of flow of nitrogen, which is used as carrier gas, is controlled by a needle valve at the column inlet.

Column packing. Acetate-washed silicone grease ⁴ was used as the static phase in all the experiments.

As inert carrier of the static phase either (1) sodium chloride crystals, or (2) Dixon rings of stainless steel gauze ⁵ were used. Preparation of the column packing took place according to either of the following methods:

(1) Stir 10 g acetate-washed silicone grease with 30 ml ethyl acetate and add 200 g sodium chloride, according to Dan. Pharm., which has passed through sieve No. 30, but which is retained on sieve No. 40. Mix thoroughly, but gently, so as not to crush the crystals. Stirring frequently heat in drying cabinet at 50°C until ethyl acetate has evaporated off.

(2) Wash 126 g Dixon rings, 1/16" × 1/16", Griffin & George Ltd., Cat. No. B 24-420, thoroughly with ethyl acetate. Add 18 g acetate-washed silicone grease to the gauze rings which must be thoroughly moistened with ethyl acetate, and distribute the grease uniformly by protracted, but gentle, stirring. Remove ethyl acetate by heating in drying cabinet at 100°C. After this procedure the grease will form a film filling the meshes of the gauze, but leaving the interior of the rings free.

COLUMN EFFICIENCY

The efficiency of a gas-liquid chromatograph may be expressed in terms of the number of theoretical plates, TP, calculated from the expression:

$$TP = 16(x/y)^2$$

where x is the distance from the starting point of the chromatogram to the centre of the elution peak, and where y is the distance between the points of intersection between the flex tangents of the elution peak and the base line.

Table 1 and 2 list the TP values obtained in a number of experiments made partly with the NaCl-column and partly with the Dixon ring-column at different temperatures and pressures. In all the experiments the rate of flow of nitrogen was 8.0 ml/min measured at atmospheric pressure and temperature.

Direct comparison between sodium chloride columns and Dixon ring columns shows the difference between the efficiencies of the two column

Table 1.

Length of column	2 m Dx	4 m Dx	4 m Dx	2 m NaCl
Temperature, °C	200	200	240	200
Outlet pressure, mm Hg	20	20	20	20
Pressure drop, mm Hg	4.5	8	10	52
Inlet pressure, mm Hg	24.5	28	30	72
TP { Methyl laurate	48	106	41	55
Methyl myristate	89	183	112	102
Methyl palmitate	137	374	121	200
Methyl stearate	205	—	226	296

Table 2.

Length of column	2 m Dx	2 m Dx	2 m Dx
Temperature, °C	200	200	180
Outlet pressure, mm Hg	20	50	20
Pressure drop, mm Hg	4.5	4	4
Inlet pressure, mm Hg	24.5	54	24
TP { Methyl caprylate	16	30	21
{ Methyl caprate	23	54	35
{ Methyl laurate	36	90	64

types to be small. A comparison based on identical outlet pressures shows the Dixon rings to be only slightly inferior, while, in case it was possible to base the comparison on identical inlet pressures, the result of the comparison would change considerably in favour of the Dixon rings, since the pressure drop in this case is so low as to enable a considerable increase in the outlet pressure. It will be seen that the number of theoretical plates increases with increasing pressure and column length and with decreasing temperature.

CALIBRATION OF APPARATUS

The apparatus has been standardized against pure methyl esters of fatty acids. The methyl esters used were purified by repeated fractional distillations on a spinning band column, and the purity was ascertained by gas-liquid chromatographic analysis of large quantities of the compounds.

The following procedure was used: temperature, pressure, rate of flow of nitrogen and detector potential were adjusted at specified values. It is essential to perform this adjustment accurately to obtain reproducible values of the areas of elution peaks without using an "internal standard". The areas found are plotted against quantities according to a double-logarithmic scale, resulting in a family of rectilinear curves, one curve for each methyl ester. The equation

$$\log A = n \cdot \log M + \log k,$$

where A is the area, M the quantity of material, while n and k are constants, applies, and the conversion factors may be found from the curves. By means of these factors the quantity of material corresponding to the measured area in the analysis of an unknown mixture can readily be calculated.

In the calibration a temperature of 180°C, a pressure of 20 mm Hg, and a rate of flow of nitrogen of 8.0 ml/min were used for methyl esters of C_8 , C_{10} , and C_{12} fatty acids. The following equations were found:

$$\begin{aligned} \text{for } C_8 \quad \log A &= 0.944 \log M + \log 9.0 \\ \text{" } C_{10} \quad \log A &= 1.045 \log M + \log 10.15 \\ \text{" } C_{12} \quad \log A &= 1.002 \log M + \log 12.35 \end{aligned}$$

For methyl esters of C_{14} , C_{16} , and C_{18} fatty acids a temperature of 200°C, a pressure of 20 mm Hg, and a rate of flow of nitrogen of 8.0 ml/min were used. The points determined are all on the same straight line, *viz*:

$$\log A = 1.041 \log M + \log 11.6$$

It was hoped that methyl esters of saturated and unsaturated fatty acids of the same chain length would prove to have identical calibration curves, since fatty acids of these types are not clearly separated under the usual experimental conditions, but occur in the same maxima. Unfortunately, experiments seem to indicate that this is not the case, since the equation for methyl oleate is

$$\log A = 1.115 \log M + \log 9.15$$

This circumstance complicates the conversion of areas into quantities by weight, since an accurate conversion cannot be made without knowledge of the ratio in which the quantities of stearic acid and unsaturated C_{18} acids are present in the methyl ester mixture to be analyzed. The problem may be circumvented by complete hydrogenation prior to the chromatography, a method which is necessary when very accurate analyses are required.

In the present work a calculation of the content of C_{18} acids in the elm seed oils based on the methyl oleate calibration has been considered to represent a sufficiently accurate compromise.

COMPOSITION OF ELM SEED OILS

The applicability of gas-liquid chromatography to fatty acid analysis has been demonstrated by the determinations of the fatty acid composition of oil from seeds of various types of elm trees. In an earlier examination ⁶ of a few types of elm seed oils C_{10} acids have been found to be present in quantities ranging from 50 to 60 %, which is very remarkable since no other plants outside the tropical zones are known to contain considerable quantities of lower fatty acids. It would therefore be interesting to find out whether oil from Danish elm types could be shown to have the same peculiar composition.

The investigation has included determinations of water and fat in the seeds. In addition refractive indices, iodine values and saponification values were determined (Table 3). The seeds were obtained from The Botanical Garden of the University of Copenhagen and from the gardens of The Royal Agricultural and Veterinary College *. The seeds were dried and the wings removed by gentle crushing and air separation.

The oil was obtained by extraction with petroleum ether. Fairly large quantities of seeds were used for the extraction because it served the double purpose of providing a determination of the fat content and material for subsequent experiments.

On the whole the examined seeds had a remarkably high oil content, considerably higher than the values given in the literature. Incidentally, the magnitude of the oil content does not, apparently, bear any relationship to the type or variety concerned, variations being presumably due to variations in growth condition.

* The authors wish to acknowledge their indebtedness to the staff of these two institutions for their kind assistance in supplying the seeds.

Table 3.

	Moisture %	Oil content %	$n_D^{25^\circ}$	Saponification value		Iodine value
				deter- mined	calcu- lated	
<i>Ulmus glabra</i> (Huds.), Botanical Garden	7.0	36.4	1.4542	281	—	18
<i>U. glabra</i> (Huds.), Vet. College	3.5	46.9	1.4523	283	287	15
<i>U. glabra</i> (Huds.), var. <i>cornuta</i> (Rehd.)	5.5	30.6	1.4565	—	267	24
<i>U. glabra</i> (Huds.), var. <i>pendula</i>	—	42.1	1.4533	284	280	16
<i>U. carpinifolia</i> (Gled.), var. <i>cornubiensis</i>	6.0	31.5	1.4553	—	282	24
<i>U. carpinifolia</i> (Gled.), var. <i>propendens</i>	6.4	17.7	1.4585	—	258	—
<i>U. procera</i> (Salisb.), var. <i>purpurea</i>	5.8	47.8	1.4536	278	279	18
<i>U. laevis</i> (Pall.)	—	34.4	1.4548	—	272	—
<i>U. fulva</i> (Michx.)	5.7	39.1	1.4537	276	275	19
<i>U. pumila</i> (L.)	6.2	30.8	1.4552	—	276	22
<i>U. campestris</i> ⁶				277.3		32.3
<i>U. americana</i> ⁶		25.6	1.4535— — 1.4574	273—275		24—25
<i>U. manshurica</i> ⁷		27	1.4552	251.4		22.5

Iodine value was determined according to AOCS, Cd 1—25, but using only 60 % of the stated quantities of material and reagents. A graphical representation of the relationship between iodine value and refractive index is given in Fig. 3. On the basis of this graph it is possible to arrive at an estimated iodine value in cases where no determination of this value has been made because the quantity of oil available was too small.

Saponification value was determined according to AOCS, Cd 3—25, likewise with reduced quantities of material and reagents.

To enable the gas-liquid chromatographic determination of the fatty acid composition, the fatty acids were converted into their methyl esters by the following method which takes into account the high solubility in water of the lower methyl esters:

About 1 g oil was dissolved in 20 ml hot methanol and a solution of 20 mg sodium methoxide in 2 ml methanol was added. The resulting solution was

Fig. 3. The relation between iodine value and refractive index.

Fig. 4. Chromatogram of methyl esters from oil *U. glabra*, 180°C.

refluxed for a few hours and allowed to stand overnight. Now 20 ml ethyl ether was added and subsequently small portions of distilled water until the solution separated into two phases. The two phases were separated, and the aqueous phase was again extracted with ether. The ether extracts were combined and an equal volume of petroleum ether was added, resulting in the separation of a considerable quantity of water. The ether mixture was now dried with anhydrous sodium sulphate, and the solvents were removed by evaporation on a steam bath. Finally the methyl ester was kept at 60°C and 15 mm Hg for about one hour.

A column temperature of 180°C in the gas-liquid chromatograph was required to obtain good separation of C_8 — C_{10} — C_{12} esters. Fig. 4 shows a characteristic chromatogram. The column efficiency is just sufficient to enable a fairly accurate determination. But the chromatogram further suggests that a determination of C_{14} — C_{16} — C_{18} esters will be difficult on account of their flattish maxima at this low column temperature. Fig. 5 shows a chromatogram run at 200°C and with a larger quantity of material. On the basis of this chromatogram a very accurate determination of C_{14} — C_{16} — C_{18} esters can be made, while C_8 — C_{10} — C_{12} esters are only just separated.

It was thus necessary to run two chromatograms for each seed sample, viz. at 180 °C with about 1.8 mg for the determination of C_8 — C_{10} — C_{12} , and at 200°C with 10—20 mg material for the determination of C_{14} — C_{16} — C_{18} . Altogether about 30 chromatograms have been run.

It is not possible by calculation to arrive at any numerical evaluation of the errors incident to the measurements made. Deviations of about 3 % have been observed in most duplicate runs, although deviations right up to 10—20 % also occur. Presumably the error in connection with the determination of C_8 and C_{12} esters is 5—10 %, while with regard to determinations of C_{10} , C_{14} and C_{16} the error is 2—5 %. For the reason referred to in the section

Fig. 5. Chromatogram of the same sample as in Fig. 4, 200°C.

on calibration the error in connection with the determination of C_{18} is considerably greater, perhaps 10–20 %. In the case of analytical samples with a more even distribution of saturated fatty acids the error may reasonably be assumed to be 1–2 %, which is greatly superior to the accuracy of other known analytical methods for similar determinations.

The gas-liquid chromatogram provides information as to the composition of the methyl ester mixture. The fatty acid composition may be obtained by calculation from the analytical results, since

$$S = \frac{E \cdot \frac{M}{14 + M}}{\sum E \cdot \frac{M}{14 + M}} \cdot 100$$

where S is the quantity of fatty acid and E the quantity of ester, both in per cent by weight, while M is the molecular weight of the fatty acid in question.

Table 4 lists the fatty acid composition for the species and varieties of elm examined. The table moreover includes a mean value which has been calculated for the purpose of enabling comparison with values given in the literature.

On the basis of the fatty acid composition found the saponification value of the corresponding elm seed oil has been calculated, the result has been included in Table 3. In the four cases where comparison has been possible, the agreement is satisfactory.

Table 4.

	Fatty acid composition, %					
	C ₈	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈
<i>Ulmus glabra</i> , Vet. College	6.2	72.4	5.3	3.3	5.0	7.8
<i>U. glabra</i> , var. <i>cornuta</i>	3.2	58.2	4.4	3.5	7.6	23.1
<i>U. glabra</i> , var. <i>pendula</i>	5.0	68.9	4.0	3.0	5.2	13.9
<i>U. carpinifolia</i> , var. <i>cornubiensis</i>	5.5	68.5	5.1	3.2	5.4	12.3
<i>U. carpinifolia</i> , var. <i>propendens</i>	1.8	54.4	3.3	3.0	6.6	30.9
<i>U. procera</i> , var. <i>purpurea</i>	3.2	68.1	5.3	4.0	7.0	12.4
<i>U. laevis</i>	0.8	66.2	5.1	2.8	5.5	19.6
<i>U. fulva</i>	3.7	64.2	6.4	4.1	7.0	14.6
<i>U. pumila</i>	3.0	65.6	3.5	3.9	8.1	15.9
Mean value	3.6	65.2	4.7	3.4	6.4	16.7
<i>U. americana</i> ⁶	5.3	61.3	5.9	4.6	2.9	20.0
<i>U. manshurica</i> ⁷	2—11	55—59	2—8	1—3	12—21	5—8

The statements in the literature with regard to a high content of C₁₀ acid as being particularly characteristic of elm seed oil have been fully confirmed.

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