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1. Development of an analytical procedure for use in genetic studies of fatty acid composition of corn oil.

Gas-liquid chromatography (GLC) has been extensively used for determining the fatty acid composition of oil of corn, flax, soybean, cotton, rape, safflower, castor bean, and other crops. Most reports indicate that the standard GLC procedure in common use requires from 15 to 25 minutes to complete the analysis of a single oil sample. Due to a lack of funds, time, and labor, development of an accurate and much more rapid method than the standard GLC procedure was necessary for studies of fatty acid composition of corn oil. The GLC procedure now in use requires about 3 minutes per sample for determination of five fatty acids (palmitic, stearic, oleic, linoleic, and linolenic). With aging of columns, the retention time of linolenic becomes shorter and sample analyses have been made in 2 minutes and 20 seconds.

A brief description of the rapid GLC procedure and some comments on past experience are as follows: The equipment consisted of an F & M Model 700 dual column chromatograph with flame ionization detectors and a Honeywell Electronik 16 recorder. Columns were 0.25 in. x 7.5 ft. packed with 15%, by weight, of stabilized diethylene glycol succinate (Analabs, Inc., Hamden, Conn.) coated on Anakrom AB 70/80 mesh solid support. Operating temperatures (C) were 235 (column), 265 to 275 (injector), and 290 (detector). Helium flow rate was 110 ml/min at 60 psi pressure. Attenuation was made for each peak in order to obtain maximum peak area for each fatty acid. Attenuation was usually 10^2 x 5 or 10 for palmitic, 10^2 x 1 or 2 for stearic, 10^2 x 10 or 20 for oleic and linoleic, and 10^2 x 1 for linolenic. Small samples (approximately 0.01 μl) were injected with a Hamilton No. 7101 1.0 μl syringe. Sample sizes may vary with different syringes; however, small samples were used so that attenuation was never higher than 10^2 x 10 for palmitic (preferably peak height no higher than 60 or 70 on the chart scale). Studies with sample sizes and the possibility of overloading the column or detector have shown that the present sample size may be increased several times without distortion of peak heights or of fatty acid composition. Our experience with another syringe (Hamilton No. 7001) has shown that a uniform sample size could not be obtained from sample to sample and that palmitic acid content was considerably over-estimated and, consequently, the other fatty acids were under-estimated. Detectors have been cleaned as frequently as once a day (a 15 min. job). Also, detectors have been used over a period of time without cleaning. The necessity for cleaning detectors is evident by the amount of baseline noise with attenuation set at 10^2 x 1.

Of course, a rapid GLC procedure is of no value unless the results are accurate and reliable. Confidence in the rapid procedure was obtained by comparing results with: (1) analysis of similar oil samples by others, (2) analysis of commercial corn oil, and (3) analysis of a reference standard with a known composition of the five major fatty acids found in corn oil. Over a period of time an average of 14 chromatograms of commercial corn oil gave the following results: 12.5% palmitic, 2.5% stearic, 28.7% oleic, 55.4% linoleic, and 1.0% linolenic. These results agree closely with the reported composition of commercial corn oil as found in the literature. Table 1 gives the results from analysis of a known standard containing 20% by weight each of five fatty acids. Comparisons were made with three column temperatures each at two helium flow rates. Time required for each procedure and recorder chart speed is also given in Table 1. The rapid procedure was as good as any of the other procedures.

Duplicate chromatograms of the same oil sample agree very closely as shown in Table 2 for 12 samples of oil. Fatty acid composition was determined by triangulation of peak areas. Only one measurement and calculation was made on each chromatogram. It is concluded that only one chromatogram per sample is necessary for genetic studies since differences (fatty acid composition) among the various oil samples are usually quite large. Duplicate chromatograms would be necessary if very small differences are to be determined.

Table 1
Average fatty acid composition of four chromatograms of a standard analyzed at three column temperatures each at two helium flow rates.

Recorder chart speed	Column temp.	Helium flow rate	Sample analysis time	Fatty acid composition (%)				
				16:0	18:0	18:1	18:2	18:3
in/min	C	ml/min	min					
0.5	180	55	25	19.6	20.1	20.7	20.2	19.4
0.5	180	110	16	20.2	20.2	20.5	20.1	19.0
0.5	200	55	15	20.1	20.1	20.6	20.4	18.9
1.0	200	110	9	19.8	20.5	20.6	20.0	19.0
1.5	235	55	4	20.5	20.2	21.6	19.9	17.9
3.0	235	110	3	19.7	20.1	20.7	20.2	19.4
Average				20.0	20.2	20.8	20.1	18.9
Standard error				0.21	0.16	0.13	0.12	0.21
Coefficient of variation (%)				2.12	1.63	1.24	1.15	2.21

Table 2
Examples of results obtained with duplicate chromatograms of 12 corn oil samples.

Sample No.		Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3
1501	A	12.46	2.67	23.05	58.30	3.51
	B	12.10	2.83	22.40	59.00	3.67
1502	A	12.76	1.47	24.33	60.69	0.75
	B	12.88	1.54	25.43	59.37	0.78
1503	A	14.74	1.78	13.81	67.15	2.52
	B	14.06	1.82	14.36	67.12	2.64
1504	A	15.15	1.01	15.76	66.56	1.52
	B	15.12	1.07	16.05	66.20	1.56
1505	A	16.22	2.49	24.23	54.09	2.98
	B	15.99	2.63	24.22	54.08	3.07
1506	A	15.32	1.60	30.45	51.85	0.78
	B	15.22	1.67	29.85	52.44	0.82
1507	A	19.17	1.58	15.28	60.94	3.03
	B	19.29	1.60	15.14	60.93	3.03
1508	A	18.43	1.11	19.54	58.78	2.13
	B	18.52	1.09	18.69	59.68	2.01
1509	A	14.88	1.82	26.26	54.03	3.00
	B	14.55	1.79	25.88	54.88	2.90
1510	A	14.99	1.53	26.74	55.82	0.92
	B	15.61	1.46	25.88	56.19	0.86
1511	A	19.33	1.85	12.91	62.65	3.27
	B	19.48	1.76	12.25	63.28	3.23
1512	A	18.85	1.16	15.44	62.35	2.20
	B	18.61	1.08	15.54	62.59	2.17

Variation is due to chromatographic equipment and, probably, mainly due to human errors in measurement of peak areas.

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2. Fatty acid composition of reciprocal crosses.

The fatty acid composition of a number of reciprocal crosses has been determined. The results for inbred lines and their reciprocal crosses are shown in Table 1. Averages are of nine kernels (fatty acid composition of oil) from each of two ears for each inbred and cross. Reciprocal crosses of GE295 and GE297 showed heterotic effect for palmitic acid. Oleic and linoleic acid composition of the reciprocal cross was similar to that of the inbred line used as the female parent. The reverse of this