Variation in Composition of Sunflower Oil From Composite Samples and Single Seeds of Varieties and Inbred Lines[1,2]

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Abstract

The seed oil of eight sunflower varieties grown at 10 locations in 1964 and 14 locations in 1964 showed highly significant differences between varieties and between stations in mean values for percentage of stearic, oleic and linoleic acids but no significant difference for palmitic acid. The same observations held for oleic and linoleic acids in three varieties common to eight stations in the two years. The only significant interaction appearing in these studies was between years and stations. Varieties requiring the same time to mature differed significantly. Oil from composite samples of inbred lines showed large differences in composition, e.g., the ranges in 56 lines grown in one season at one location were: palmitic 4.7–8.2%; stearic 1.7–9.1%; oleic 13.9–40.3%; and linoleic 47.9–76.4%. Single seeds within inbred lines also showed striking variation. The greatest variation occurred in lines inbred for one to three generations and the least in lines inbred for eight to nine generations. Pairs of lines with identical or similar flowering date differed significantly in mean values of all four acids. Variation between seeds within varieties were relatively narrow in Armavirec and Advent, but wide in Peredovik where the range was: palmitic 4.5–9.4%; stearic 2.5–12.4%; oleic 14.8–46.4%; and linoleic 34.3–75.5%. The results show that genetic control of oil quality, independent of flowering or maturity date, exists in sunflowers. The wide range in composition suggests that altering oil quality in the crop by breeding is a practical objective.

Introduction

This paper reports the percentage of fatty acids in the oil from varieties and inbred lines of sunflowers. Recently, atypical oils have been found within species of oilseed crops. Examples include the strains with no erucic acid in rapeseed *Brassica napus* (11), turnip rape *(B. campestris)* (2) and the oils of low iodine number in safflower *(Carthamus tinctorius)* (5,8). Intervarietal differences in iodine number and fatty acid composition have been reported in sunflowers in Europe (6) and the United States (7). These examples suggested that more extensive study should be carried out in sunflowers to assess the prospects of breeding for specific types of oil in the crop.

Though sunflower oil is recognized as a high quality edible and cooking oil it has some undesirable characteristics. Because it is a semi-drying oil it polymerizes when heated during production of French-fried potatoes or similar food products. This leads to objectionable gumming of the walls of the vessels and of surfaces of the stirring equipment. Reduction of the iodine number or drying quality may overcome this objection and provide broader markets for the oil. In contrast some individuals in the paint industry believe that the oil would find wider use in that industry if the iodine value were increased.

The iodine number, or degree of unsaturation, in sunflower oil varies inversely with temperature during development of the seed (1,4,7). Thus, in breeding for oil quality in the crop, differences between varieties or selections must be considered in relation to the mean temperatures after blooming.

Experimental Procedures and Data

Eight varieties were grown at 10 locations across Canada in 1963 and 14 locations in 1964. Three varieties were common at eight locations in the two seasons. The percentages of palmitic, stearic, oleic and linoleic acid were determined by gas-liquid chromatography (GLC) in the oil from the open pollinated seed using duplicate samples from the entries of each test. Variance analyses were made on the data from each of the two years and on the combined data for the three varieties common in the two seasons. The estimates of the variance components for varieties and their interactions with locations and years were determined as outlined by Rasmussen and Lambert (9), and the appropriate degrees of freedom for the F tests were computed according to Satterthwaite (10).

The second portion of the study involved composite samples of inbred lines and single seeds of inbred lines

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TABLE I

Mean Per Cent Fatty Acid in Oil of Sunflower Varieties in 1963 and 1964 Co-operative Tests

Variety	Palmitic		Stearic		Oleic		Linoleic		Days to Mature	
	1963	1964	1963	1964	1963	1964	1963	1964	1963	1964
Advent	6.04	6.08	5.07	3.76	18.30	18.00	70.62	72.08	124	124
Commander	5.96	6.01	4.77	3.18	18.17	17.74	71.13	72.98	124	125
Peredovik	6.14	6.08	5.50	3.72	16.62	16.44	71.77	73.68	124	124
VNIIMK 8931	6.40	.....	5.70	.....	16.01	.....	71.92	.....	125	.....
Smena	6.01	.....	5.59	.....	16.64	.....	71.81	.....	125	.....
Armavirec	.....	5.98	.....	4.31	.....	18.09	.....	71.56	.....	110
Ienissei	.....	6.15	.....	3.96	.....	17.74	.....	72.07	.....	110
Variance components										
Varieties	.010	.017	.133[b]	.084[b]	.624[b]	.460[b]	.139[a]	.492[b]	.....	.....
Locations	.138[b]	.510[b]	.527[b]	.189[b]	2.868[b]	9.913[b]	2.648[b]	11.593[b]	.....	.....
Var. X loc.	.009	.135[b]	.041	—.095	— .110	— .185	.23	.355	.....	.....
Error	.186	.170	.229	.610	1.217	3.500	1.248	4.640	.....	.....

[a] Significant at P = .05.
[b] Significant at P = .01.

TABLE II

Mean Per Cent Fatty Acid in Three Sunflower Varieties at Eight Locations in Two Years

Variety	Palmitic	Stearic	Oleic	Linoleic	Days to mature
Advent	6.14	4.37	17.75	71.55	124
Commander	5.95	3.99	17.64	72.26	125
Peredovik	6.15	4.57	16.25	72.85	124
Variance components					
Years	−.025	1.24[b]	−1.400	− .643	.....
Locations	−.064	.009	−2.520	−4.590	.....
Years X loc.	.287[a]	.298[b]	4.350[b]	4.663[b]	.....
Varieties	.002	.073	.734[b]	.448[b]	.....
Var. X loc.	.035	− .043	− .283	− .368	.....
Var. X yrs.	.000	− .021	− .093	− .112	.....
Var. X yrs. X loc.	−.175	− .010	− .380	−1.230	.....
Error	.695	.290	2.340	4.960	.....

[a] Significant at P = .05.
[b] Significant at P = .01.

and varieties. The lines had been inbred for one to nine generations and were composited when they appeared uniform for agronomic features such as plant height, maturity, disease resistance and seed color, shape and size. The fatty acid content was determined in oil from composite samples of 50 seeds of 129 inbred lines. After the data on the composite samples were obtained, seven lines were selected which, as a group, showed a range in fatty acid composition. The oils from the upper portions of 42 single seeds of each of the seven lines, 199 seeds of the variety Peredovik, and 108 seeds of each of the varieties Armavirec and Advent were then analyzed. Peredovik and Armavirec are open pollinated varieties of Russian origin and Advent is a rust-resistant, top cross hybrid of Canadian origin. All analyses in this part of the study were done by GLC.

The single seed analyses were made on a portion of the seed while the remainder containing the embryo was saved for growing into a plant. One quarter to one third of the upper end of each seed was excised with a jeweller's hacksaw and the hull was removed from the portion used for analysis. This portion was placed in a 50 ml Erlenmeyer with a 19/38 T/S joint; 10 ml of a solution of methanol-benzene-acetyl chloride (80:15:5 v/v/v) was added and the mixture was refluxed under an air condenser for 40 min to extract the oil and form the methyl esters. A drop of indicator (bromphenol blue), 0.75 g sodium carbonate and 20 ml of water was added to the solution which was shaken and cooled to room temperature. The methyl esters were extracted by adding 10 ml of low boiling petroleum ether (Skellysolve "F") to the flask. Water was added to bring the solvent layer into the neck of the flask; the petroleum ether layer was transferred to a round bottom flask with a conical tip using a pipette and the solvent was evaporated. GLC analyses were made on a unit equipped with a T/C detector. The column was 5 ft × 3/16 in. OD copper filled with a packing of succinate-ethylene glycol on acid washed Chromosorb W (60–80 mesh) in the ratio of 1:6 (w/w). The unit was operated at 205 C with a flow rate of 50 ml/min of helium gas. Five minutes were

TABLE III

Range in Per Cent Fatty Acids of Oil From Bulked Seed of Inbred Lines

Year	No. of lines	Palmitic		Stearic		Oleic		Linoleic	
		Low	High	Low	High	Low	High	Low	High
1959	56	4.7	8.2	1.7	9.1	13.9	40.3	47.9	76.4
1961	31	5.8	7.9	2.6	9.7	13.4	34.7	49.1	75.3
1963	12	5.2	8.1	2.5	7.5	13.0	34.5	54.6	75.4
1964	17	5.0	8.6	2.7	8.4	9.2	18.3	68.8	79.6
1965	13	5.2	7.0	1.7	4.3	9.1	19.9	76.6	81.8

TABLE IV

Per Cent Fatty Acid in Oil From Bulked Seed of Some Inbred Lines

Line	Date of bloom	Palmitic	Stearic	Oleic	Linoleic
CM 32	30–7–59	7.3	4.2	16.5	72.0
CM 177	30–7–59	6.9	4.9	40.3	47.9
CM 150	31–7–59	6.0	4.9	20.5	68.6
CM 132	31–7–59	7.0	6.9	35.9	50.2
CM 13	4–8–59	7.6	3.3	17.8	71.3
CM 83	4–8–59	5.5	8.7	33.1	52.7
CM 255	22–7–61	7.2	4.2	13.4	75.2
CM 79	22–7–61	6.5	9.7	34.7	49.1
CM 302	24–7–63	7.4	2.5	13.0	77.1
CM 308	6–8–63	7.3	7.0	30.5	55.2

required for each analysis and the peak areas were measured with an electronic integrator.

Table I shows representative mean values for varieties in each of the two years from the cooperative tests and the results of the variance analyses. Table II provides data for the three varieties grown at the seven stations in the two seasons.

Within the separate years the variances for varieties exceeded P = .01 for stearic, oleic and linoleic acids, except for linoleic in 1963, where the value exceeded P = .05 (Table I). All variances for locations were above P = .01 and were much greater than the values for varieties. The variances for the interaction of varieties and locations were not significant except for palmitic acid in 1964.

Linoleic acid exceeded 70% and was higher in 1964 than in 1963 (Table 1). The higher level in 1964 for Advent, Commander and Peredovik was accompanied by a lower stearic acid content. Values for oleic acid were similar in the two years.

In the data for the three varieties grown in two years the variances for varieties exceeded P = .01 for oleic and linoleic acids but were not significant for the two saturated acids (Table II). The greatest range in oleic and linoleic acids occurred between Advent and Peredovik which required the same number of days to mature. None of the variances for locations over the two years was significant, but the components for the interactions of years and locations exceeded P = .05 for palmitic acid and P = .01 for the other three acids. None of the varietal interactions, was significant. The variance for years for stearic acid was highly significant which reflects the difference between the levels of this acid in 1963 and 1964 recorded in Table I.

The oil from the bulk samples of the inbred lines showed a wide range in the proportion of the different acids (Table III). The ratio between the low and high values for palmitic approached 1:2 whereas for stearic it reached 1:5. The high linoleic content of the lines in 1964 and 1965 reflects cool seasons.

Some pairs of inbred lines flowered on the same date but had greatly different proportions of acids in their oil (Table IV). Another pair of lines, CM302 and CM308, differed by 11 days in blooming date and also differed greatly in oil quality. In this pair, CM302 bloomed earlier and had the lower level of linoleic acid. The mean temperature was 70 F during the 30 days after CM302 bloomed. It was 67 λ F in the 30 days after CM308 bloomed.

Individual seeds of Peredovik and of some inbred lines showed a wide range in per cent fatty acids (Table V, Fig. 1 and 2). The range among seeds of the inbreds CM198 and CM203 was much narrower than in some of the other material. These two lines had been inbred for eight and nine generations, respectively. Consequently they approached genetic

TABLE V

Per Cent Fatty Acids in Oil From Single Seeds of Varieties and of 1959 Inbred Lines and "t" Values for Differences Between Lines

	Years inbred	Date bloom	No. of seeds	Palmitic		Stearic		Oleic		Linoleic	
				Mean	Range	Mean	Range	Mean	Range	Mean	Range
Peredovik	..	....	199	6.2	4.9	5.7	9.9	24.4	21.6	63.7	41.2
Advent	..	....	108	6.4	4.0	3.7	6.2	15.2	18.3	74.8	18.6
Armavirec	..	....	108	6.4	3.7	4.4	5.7	15.9	9.6	73.4	12.2
CM 83	2	Aug. 4	42	6.4	2.6	9.2	11.1	32.7	15.9	51.8	26.5
CM 88	1	Aug. 6	42	7.1	2.0	3.2	4.6	13.1	8.5	76.7	16.7
CM 132	3	July 31	42	7.9	3.0	6.4	4.9	33.9	22.5	51.9	23.5
CM 142	2	....	41	6.7	2.9	4.6	4.0	16.8	12.7	72.0	16.1
CM 177	3	July 30	42	8.0	5.0	4.5	3.0	40.7	38.9	46.8	40.0
CM 198	9	July 30	42	9.3	7.0	1.4	1.9	27.6	15.2	61.7	12.4
CM 203	8	Aug. 7	42	6.7	2.8	2.9	1.6	18.4	12.3	72.0	10.2
Difference				"t" Values[a]							
CM 83 vs 88				4.0	....	19.6	....	34.0	.....	31.8	.....
CM 88 vs 203				2.8	....	1.9	....	9.9	.....	8.3	.....
CM 177 vs 132				0.4	....	9.9	....	5.7	.....	4.1	.....
CM 177 vs 198				4.6	....	26.8	....	12.1	.....	13.0	.....
CM 132 vs 198				5.8	....	32.3	....	6.9	.....	10.7	.....

[a] Value required for significance at P = .05 is 2.0, at P = .01 is 3.6.

homozygosity and the range they exhibited may be attributed to environment.

Some of the inbred lines grown in 1959, in which oil of individual seeds was analyzed, had similar or identical flowering dates. The "t" values for the mean differences between such lines exceeded values required for significance at P = .01 (Table V), with rare exceptions. The values for the differences in palmitic acid were noticeably lower than for the other three acids.

The relationships between the acids as measured by correlation coefficients are given in Table VI. The inverse relation between oleic and linoleic acids was the most consistent and strongest association. There was evidence of negative association between stearic and linoleic acids; most coefficients exceeded P = .01 and were consistent in sign except for CM203. A relationship between stearic and oleic acid was suggested; all coefficients were positive except for CM203 and eight of the 13 exceeded either P = .05 or P = .01. The associations of palmitic with the other acids were weak as shown by small coefficients with inconsistent sign.

Discussion

These studies show that genetic control of oil quality occurs in sunflowers, as judged by the significant differences in the composition of the oils from the varieties in the cooperative tests; by the wide range exhibited in bulk samples of inbred lines; by the large "t" values for the differences between inbred lines in which single seeds were analyzed; and by the narrow intraline variability of long term, or homozygous inbreds such as CM198 and CM203 compared to the open pollinated variety Peredovik and shorter term inbreds.

Of equal importance to the breeder is the evidence that genetic control of oil quality operates independently of environment. Several pairs of lines with identical flowering dates and thus identical environments, specifically temperatures, during development of the seed differed markedly in oil quality. The "t" values for the differences between such pairs of lines, in which individual seeds were analyzed, greatly exceeded the level for significance at P= .01. In the pair of lines CM302 and CM308 the later blooming line CM302 had much lower linoleic acid, or degree of unsaturation, but it developed seed during a period of lower temperature than for CM308. Considering the effect of temperature on unsaturation observed by others (1,4,7) the lower degree of unsaturation would have been expected in CM308. Genetic differences in control of oil quality evidently overcame the usual effect of environmental temperatures in this instance.

In rape, safflower and flax (3,8,12) composition of oil is controlled by the genotype of the seed rather

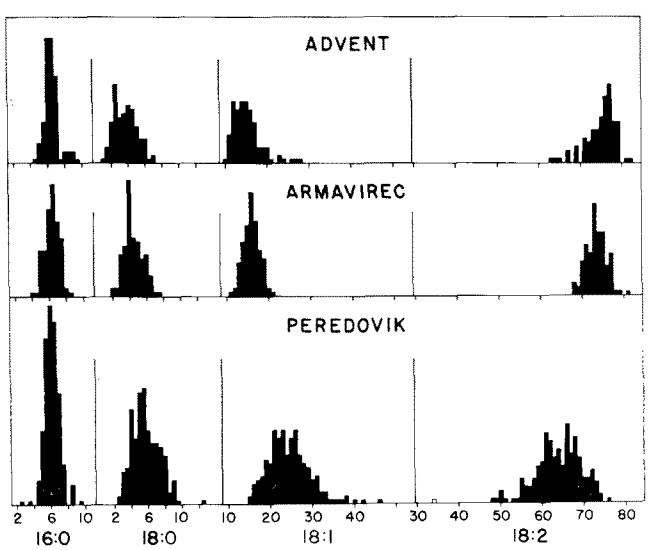

FIG. 1. Variability in fatty acid composition of sunflowerseed oil from single seeds of commercial varieties (16:0, palmitic; 18:0, stearic; 18:1, oleic; and 18:2, linoleic).

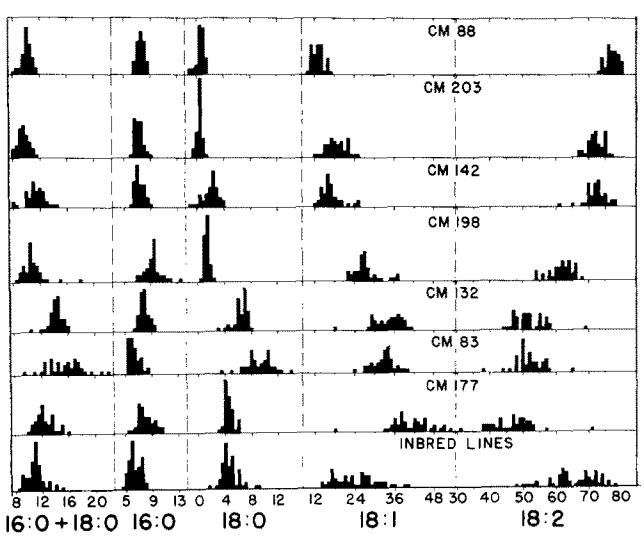

FIG. 2. Variability in fatty acid composition of sunflowerseed oil from inbred lines and single seeds of seven inbred lines (16:0, palmitic; 18:0, stearic; 18:1, oleic and 18:2, linoleic).

TABLE VI

Simple Correlation Coefficients Between Pairs of Fatty Acids in Inbred Lines and Varieties

Material	O-L	S-L	S-O	P-L	P-O	P-S
Bulk Inbreds	−.97[b]	−.55[b]	.45[b]	.13	−.20	.39[a]
CM 83	−.82[b]	−.71[b]	.21	−.23	−.24	.50[b]
CM 88	−.91[b]	−.48[b]	.22	−.13	−.16	−.08
CM 132	−.98[b]	−.55[b]	.39[a]	.55[b]	−.62[b]	−.39[a]
CM 142	−.95[b]	−.70[b]	.50[b]	−.39[a]	.23	.09
CM 177	−.98[b]	−.48[b]	.36[a]	.12	−.31[a]	.07
CM 198	−.89[b]	−.01	.11	.11	−.33[a]	−.39[a]
CM 203	−.96[b]	.17	−.34[a]	.34[a]	−.56[b]	.29
CM 83–203	−.98[b]	−.54[b]	.41[b]	−.19	.17	−.33[b]
Armavirec	−.80[b]	−.48[b]	.02	−.31[b]	−.05	.02
Advent	−.91[b]	−.37	.03	.02	−.22	−.12
Peredovik	−.96[b]	−.65[b]	.44[b]	.29[b]	−.41[b]	−.21
Overall	−.98[b]	−.53[b]	.40[b]	−.20	.15	−.28[b]

[a] Significant at P = .05.
[b] Significant at P = .01.
O—Oleic; L—Linoleic; S—Stearic; P—Palmitic.
Degrees of Freedom—Bulk Inbreds—127; CM 83, 88, 132, 177, 198, 203—40; CM 142—39; CM 83–203—291; Armavirec, Advent—106; Peredovik—197; Overall—835.

than by the genotype of maternal plant. Assuming that the same holds for sunflowers it is of interest that significant differences between varieties existed in the cooperative tests, particularly between varieties requiring the same time to mature. The crop is largely cross pollinated; consequently the male parent would have contributed uniformly to the genotype of the seed. Only the female parent would have contributed to genotypic differences between varieties. This evidence that variation in the genotype from only one parent can affect oil quality further supports the belief that oil quality is under genetic control.

The significant interaction of years with locations in the cooperative tests indicates that the environments differed among individual tests. In spite of this the comparative performance of varieties in the different tests remained constant as shown by lack of significant variances for the interactions of varieties with years and with locations. Testing at a single or a few locations would appear sufficient to evaluate the relative quality of oils from different breeding material.

Breeding for different levels of stearic, oleic, and linoleic acids appears feasible considering the wide range shown by the bulked samples of inbred lines and by the single seeds of material such as Peredovik and the lines which had been inbred for one to three generations. The generally lower ranges for palmitic acid indicate that selection for different levels of this acid will be more difficult.

The strong negative association of oleic and linoleic acids is similar to that occurring in safflower (8). Clearly these two acids belong to the same synthetizing system; selection for different levels of one will inevitably change the levels of the other. Both these acids showed some association with stearic so that selection for either may also affect the level of stearic. Because palmitic showed no consistent association with any of the other three acids breeding work with them would not affect the level of palmitic.

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