

Genetic and Environmental Variation in Oil Content and Fatty Acid Composition of Oats

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ABSTRACT

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Oil content and fatty acid composition of oat varieties and breeding lines were studied in nine trials at six locations in two years. All samples contained palmitic, stearic, oleic, linoleic, linolenic, and eicosenoic acids. Myristic, palmitoleic, arachidic, and erucic acids were found in most samples at low concentrations. Low growth temperature increased the overall lipid synthesis; 65.2% of the variation in oil content could be explained by mean temperatures of growing time of oat varieties or lines. Low temperature increased the synthesis of oleic and linoleic acids and decreased the concentrations of palmitic, stearic, and erucic acids. Small but significant differences in oil content and fatty acid composition were

found between the oat varieties studied. Lower linoleic acid concentration was associated with higher oleic acid concentration. Especially high negative correlations were found between oleic and linoleic acid contents in different trials ($r = -0.755$, $P < 0.05$; to $r = -0.911$, $P < 0.001$), which can be explained by the synthesis of linoleic acid by successive desaturation from oleic acid. Significant negative correlations prevailed, also, between oleic and linolenic acid ($r = -0.401$ to -0.832 , $P < 0.01$) and stearic and eicosenoic acid ($r = -0.019$ to -0.833 , $P < 0.001$) concentrations. The correlation coefficients between linoleic and linolenic acid concentrations were positive ($r = 0.278$ to 0.714 , $P < 0.05$).

Oats (*Avena sativa* L.) contain a considerable amount of oil in their seeds compared to other cereal crops, about 3–12% of dry matter (Brown and Craddock 1972).

The fatty acid composition of oat oil is desirable from both technological and nutritional standpoints. It contains unsaturated fatty acids, such as oleic (18:1), linoleic (18:2), and linolenic (18:3) acid, and saturated fatty acids such as myristic (14:0), palmitic (16:0), and stearic (18:0) acid. Oleic, linoleic, and palmitic acids are predominant (Hammond 1983, Youngs 1986). Linoleic and linolenic acids are essential fatty acids in mammalian nutrition. Palmitic acid increases oil stability against peroxidation whereas linolenic acid causes oil instability (Hammond et al 1972, Thro et al 1983). The natural peroxidation of linolenic acid results in hydroperoxides that are toxic to mammals and cause poor oil flavor (Mounts et al 1978). The favorable fatty acid composition of oat oil thus increases the nutritional value of oats for human and animal nutrition.

Oats are extensively cultivated in Finland because of their adaptability to cultivation in acid peat soils, which are abundant. Oats are used mainly as feed in animal husbandry. However, the use of oats by the Finnish food industry has also increased in the production of flakes, biscuits, special breakfast products, and in breadmaking.

The oil content and fatty acid composition of the most commonly cultivated Finnish oat varieties and breeding lines were studied in order to determine possible genetic and environmental variation in these quality characters under Finnish conditions.

MATERIALS AND METHODS

Plant Material and Its Cultivation

The oil content and fatty acid composition of oat varieties and breeding lines in nine official oat trials were analyzed. The oat trials were established at research institutions and research stations (Table I) of the Agricultural Research Centre of Finland during 1986 and 1987 using official experimental designs (Table II) (Cochran and Cox 1957). Plot size was 12.5 m² and there were four replications in these trials.

Environmental Conditions

The year 1986 was rather normal, whereas 1987 was characterized by much lower temperatures. Two trials were established at the North Ostrobothnia Research Station; one on

fine sand soil and the other on peat soil. Peat soils are also usually colder growth media than are mineral soils. Being old swamps, peat soils are located at low elevations and have a colder microclimate than that of higher elevations.

Analyses of Oil Content

Oil content and fatty acid analyses were performed on whole milled grain including the hull and bran. Oil contents were analyzed by nuclear magnetic resonance (NMR) employing Newport Analyser Magnet Type 10 equipment. Oat samples were dried overnight at 105°C. The NMR equipment was calibrated by a 6.191-g rape oil ampule adjusting it to give an oil percentage of 41.3. Samples (15 g) of oat seed were measured for oil contents, five samples per variety or line. The two most divergent results were eliminated and the mean of three samples was used as the true value for oil content.

Analyses of Fatty Acid Composition

For the determination of fatty acids, homogenized 5-g samples were extracted using a mixture of chloroform and methanol (2:1). After filtration, the solution was washed with water, the phases separated, and the methanol-water phase discarded. The chloroform solution was evaporated to dryness using nitrogen flow. Residual fat was saponified by heating for 7 min in 2.0 ml of 0.5N NaOH at 85°C. Fatty acids were esterified by heating in 2 ml of 10% BF₃-CH₃OH solution for 12 min followed by extraction into hexane. The esterified fatty acids were kept in a nitrogen atmosphere at +4°C until analysis. All the reagents used were of reagent grade purity. The fat extraction method used was based on the method of Folch et al (1957) and the

TABLE I
Oat Trial Locations

Name of Research Facility	Abbreviation	Location	Situation
South Savo Research Station	ESA	Mikkeli	long. 27° 13' E lat. 61° 40' N
Häme Research Station	HÄM	Pälkäne	long. 24° 13' E lat. 61° 20' N
Institute of Plant Breeding	KJL	Jokioinen	long. 23° 29' E lat. 60° 49' N
Kymenlaakso Research Station	KYM	Anjala	long. 26° 48' E lat. 60° 43' N
North Ostrobothnia Research Station	PPO	Ruukki	long. 25° 05' E lat. 64° 40' N
Satakunta Research Station	SAT	Kokemäki	long. 22° 14' E lat. 61° 17' N

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esterification of fatty acids was based on the method of Metcalfe and Schmitz (1961).

Fatty acid methyl esters were determined using a Perkin-Elmer Model Sigma 1 gas-liquid chromatograph equipped with a Perkin-Elmer Model Sigma 115 Integrator. A silica capillary column (FFAP, 30 m × 0.22 mm i.d., film thickness 0.2 μm) was used for separation of the methyl esters. Analytical conditions used were as follows: detection: flame ionization detector (FID); split ratio, 20:1; injector temperature, 240°C; detector temperature, 260°C; initial oven temperature, 150°C; step 2 oven temperature, 190°C (1 min, ramp rate 15°C/min, from 150 to 190°C); step 3 oven temperature, 210°C (1 min, ramp rate 2°C/min, from 190 to 210°C); final oven temperature, 240°C (15 min, ramp rate 10°C/min, from 210 to 240°C); nitrogen flow rate, 0.76 ml/min; peak integration tolerance limit, 0.15%.

A mixture of fatty acid methyl ester standards (Nu Check Prep., Elysian, MN) was used for identification and quantitation.

RESULTS AND DISCUSSION

Environmental Effects on Oil Content

The mean oil content of the trials varied from 6.1 to 7.8% (Table III). The highest mean oil contents were from the 1987 trials and from the North Ostrobothnia Research Station in peat soil in 1986. The yields from the year 1987 were rather high, and the average growing times of varieties and lines were much longer for the lower mean temperature of the growing period (Table IV). Protein contents of trials were lower and average height of plants was higher in 1987 than in 1986. The longer mean growing time was characteristic also of the trial in peat

TABLE II
Sowing Times and Fertilization Variables

Year	Trial Location ^a	Date of Sowing	Fertilization (kg/ha)			Soil Type	Experimental Design
			N	P	K		
1986	HÄM	May 21	96	42	78	fine, sandy silt	randomized blocks
	KJL	May 21	80	35	65	mull ^b	rectangular lattice
	KYM	May 19	80	35	65	mull	partially balanced lattice
	SAT	May 16	96	42	78	fine sand	rectangular lattice
	PPO	May 30	80	35	65	very fine sand	randomized blocks
	PPO	June 3	80	35	65	peat	randomized blocks
1987	ESA	May 20	80	35	91	fine sand	randomized blocks
	KJL	May 20	96	42	78	fine sand	rectangular lattice
	KYM	May 25	80	35	65	mull	partially balanced lattice

^aAbbreviations from Table I.

^bContains 20–40% humus.

TABLE III
Means and Standard Deviations of Oil Content and Fatty Acid Concentrations of Some Oat Trials

Year	Trial Location ^a	No. of Varieties	Fatty Acids %															
			Oil %		Palmitic Acid		Stearic Acid		Oleic Acid		Linoleic Acid		Linolenic Acid		Eicosenoic Acid		Erucic Acid	
			\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
1986	HÄM Pälkäne	6	6.2	0.20	17.4	0.31	1.1	0.13	38.5	0.89	39.1	0.91	2.0	0.12	0.9	0.07	0.3	0.03
	KJL Jokioinen	7	6.6	0.28	16.1	0.20	1.4	0.11	40.5	0.78	38.6	0.84	1.6	0.08	0.8	0.05	0.5	0.08
	KYM Anjala	4	6.1	0.10	17.3	0.93	1.3	0.24	37.2	0.28	40.6	1.06	1.9	0.13	0.9	0.06	0.4	0.06
	SAT Kokemäki	9	6.4	0.16	17.2	0.46	1.3	0.24	38.9	1.07	39.2	0.95	1.7	0.11	0.8	0.06	0.3	0.04
	PPO Ruukki ^b	6	6.5	0.41	15.4	0.58	0.9	0.16	37.7	0.82	42.5	0.69	2.0	0.12	0.9	0.05	0.4	0.03
	PPO Ruukki ^c	5	7.3	0.40	14.4	0.72	0.8	0.18	40.3	1.52	41.0	1.11	1.8	0.15	1.0	0.09	0.5	0.04
1987	ESA Mikkeli	16	7.8	0.67	13.2	0.48	1.1	0.15	40.6	1.46	42.1	1.16	1.9	0.26	0.9	0.07	0.2	0.10
	KJL Jokioinen	4	7.7	0.26	13.7	0.30	1.0	0.24	42.1	1.07	40.9	1.02	1.3	0.16	0.7	0.12	0.4	0.13
	KYM Anjala	14	7.1	0.33	13.9	0.49	1.0	0.22	41.4	1.15	41.4	1.28	1.3	0.12	0.7	0.06	0.1	0.10

^aAbbreviations from Table I.

^bSoil type: very fine sand.

^cSoil type: peat.

TABLE IV
Means of Grain Yield, Height, Growing Time to Yellow Ripening, Protein Content, and Growing Temperature of Varieties and Lines in Different Trials

Year	Trial Location ^a	No. of Varieties	Mean Grain Yield (kg/ha)	Mean Height (cm)	Mean Growing Time Days	Mean Temperature of Growing Time (°C)		Mean Protein Content (%)
1986	HÄM Pälkäne	6	5,090	82	83	15.8		15.6
	KJL Jokioinen	7	3,600	77	88	15.3		12.0
	KYM Anjala	4	2,650	52	89	18.1		18.0
	SAT Kokemäki	9	3,360	51	84	15.1		16.9
	PPO Ruukki ^b	6	4,930	86	98	13.8		13.1
	PPO Ruukki ^c	5	2,660	81	112	12.5		12.3
1987	ESA Mikkeli	16	4,240	106	141	11.1		11.1
	KJL Jokioinen	4	6,600	117	128	11.7		11.4
	KYM Anjala	14	4,950	96	115	12.6		10.3

^aAbbreviations from Table I.

^bSoil type: very fine sand.

^cSoil type: peat.

soil at North Ostrobothnia in 1986.

When the mean temperature (x) of the growing time for a variety or line was used as the independent variable, the oil content (y) of oats could be explained by the linear regression equation $y = 10.94 - 0.294x$, $r^2 = 65.28\%$, $n = 71$. Thus, 65.28% of the variation in oil content could be explained by temperature. Beringer (1967) reported that low growth temperature (12°C) increases the oil content of oats compared to higher growth temperature (30°C). Welch (1975) also reported that low temperature increases lipid synthesis of oats.

Fatty Acid Composition

Six fatty acids were found in every oat sample: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and eicosenoic (20:1) acids (Table III). Myristic (14:0), palmitoleic (16:1), arachidic (20:0), and erucic (22:1) acids were found in small concentrations in most of the oat varieties and lines (Table V). In most previous studies only myristic, palmitic, stearic, oleic, linoleic, and linolenic acids were found (Forsberg et al 1974, Karow et al 1984, Saastamoinen 1987). Frey and Hammond (1975) found small amounts (<0.1%) of lauric, palmitoleic, and arachidic acids in oat oil. The separation capability of the packed columns used in previous studies was not as good as that of the modern capillary columns used in this study. Sahasrabudhe (1979) found 20:1 to 20:5 fatty acids in small amounts (0.5–3.0% in all) and traces of behenic, erucic, and lignoseric acids, but the amounts of individual fatty acids were not specified.

Environmental Effects on Fatty Acid Composition

The correlation coefficients between oil content and different fatty acid concentrations within various oat varieties are shown in Table VI. When oil content was increased, palmitic, stearic, and erucic acid concentrations decreased, and oleic and linoleic acid concentrations increased. The correlation coefficients between oil content and linoleic and eicosenoic acids were not significant.

It seems to be quite certain that the variation in temperature affected lipid synthesis. The increase in oil content caused by lower temperature increased oleic and linoleic acid concentrations. Beringer (1971a,b) found that low growth temperature (12°C) increases the oil content of oats and results in greater unsaturated fatty acid content compared with higher growth temperature (28°C). Stearic and palmitic acid concentrations decrease sharply at lower growth temperatures whereas oleic and linoleic acid concentrations increase. Also Welch (1975) found that low temperatures cause a higher synthesis of unsaturated fatty acids in oats.

Differences Between Oat Varieties and Correlations Between Different Fatty Acids

Major differences were not found among the different oat varieties and breeding lines when tested by paired t statistics (Table V). The oat variety Puhti had the highest mean oil content, and the breeding line, Jo 1069, the lowest. Some small significant differences in fatty acid composition were found among different varieties (Table V). Compared with Puhti, the other oats showed an inverse relationship between oleic and linoleic acids; if oleic acid content was higher, linoleic acid concentration was lower, and vice versa. The change in oil content was not always associated with the same type of change in fatty acid composition, however.

The synthesis of linoleic acid from oleic acid by successive desaturation explains the high negative correlations found between concentrations of these acids (Table VII) (Vijay and Stumpf 1970, Cherif et al 1975, Slack et al 1979). In soybean (*Glycine max* (L.) Merr.), selection for higher oleic acid content has simultaneously decreased linoleic acid concentration (Wilson et al 1981, Burton et al 1983). The high oleic acid mutant of soybean, A5, has decreased rates of oleic acid desaturation of oleoyl-phosphatidylcholine compared with the normal genotype (Martin and Rinne 1986). High negative correlations between these fatty acid contents in oats have been found in previous studies, too (Forsberg et al 1974, Thro et al 1985, Saastamoinen

TABLE V
Mean Oil Content and Fatty Acid Composition of Some Oat Varieties and Breeding Lines Compared With Puhti, the Standard Variety

Variety	No. of Trials	Oil Content (%)	Fatty Acids (%)									
			Myristic Acid (14:0)	Palmitic Acid (16:0)	Palmitoleic Acid (16:1)	Stearic Acid (18:0)	Oleic Acid (18:1)	Linoleic Acid (18:2)	Linolenic Acid (18:3)	Arachidic Acid (20:0)	Eicosenoic Acid (20:1)	Erucic Acid (22:1)
Puhti, standard	9	7.2	0.1	15.4	0.1	1.2	39.8	40.6	1.7	0.1	0.8	0.3
Deviation from Puhti ^a												
Veli	8	-0.3*	±0.0	±0.0	±0.0	-0.2**	+1.5**	-1.5***	±0.0	±0.0	+0.1*	±0.0
Stil	6	-0.6*	±0.0	-0.3	±0.0	-0.1	+0.4	-0.1	±0.0	±0.0	±0.0	±0.0
Jo 1068	7	-0.5*	±0.0	±0.0	+0.1	-0.4***	-1.1	+1.4**	±0.0	-0.1	+0.1*	±0.0
Jo 1069	7	-0.6*	+0.1	+0.3	+0.1	-0.2*	-0.8**	+0.3	+0.2*	±0.0	+0.1	±0.0
Jo 1099	5	-0.4	±0.0	+0.2	±0.0	-0.3**	-0.6	+0.3	+0.2	±0.0	+0.1*	±0.0
Ryhti	5	-0.1	±0.0	-0.2	±0.0	+0.1	+1.5***	-1.3**	-0.1	±0.0	±0.0	+0.1
Nasta	3	-0.3	±0.0	-0.5	±0.0	+0.2	+0.8	-0.6	±0.0	±0.0	+0.1	+0.1
Hja's Vouti	3	-0.1	+0.0	+0.1	±0.0	-0.2	-0.1	+0.2	-0.1	±0.0	±0.0	±0.0

^aSignificance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. 5.

TABLE VI
Correlation Coefficients Between Oil Content and Fatty Acid Contents In Some Oat Varieties

Variety	No. of Trials	Correlation Coefficient (r)						
		Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Eicosenoic Acid	Erucic Acid
Puhti	9	-0.912***a	-0.745*	0.777*	0.708*	-0.518	-0.171	-0.225
Veli	8	-0.831*	-0.602	0.881**	0.200	-0.423	-0.145	-0.528
Stil	6	-0.885*	-0.408	0.760	0.568	0.000	0.279	-0.631
Ryhti	5	-0.994***	-0.788	0.865	0.951*	-0.039	-0.510	-0.198
Jo 1068	7	-0.866*	-0.396	0.830*	0.334	-0.363	0.000	-0.518
Jo 1069	7	-0.919**	-0.881**	0.843*	0.253	-0.291	0.227	-0.415
Jo 1099	6	-0.956**	-0.694	0.701	0.479	0.061	0.723	-0.882*

^aSignificance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

TABLE VII
Correlation Coefficients (*r*) Between Fatty Acid Concentrations Within Four Trials at Four Locations

Fatty Acid/ Growth Trial ^a	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Eicosenoic Acid	Erucic Acid
Palmitic acid						
ESA (<i>n</i> = 16)	-0.552 ^b	-0.465	0.088	0.632 ^{**}	0.403	0.027
KJL 1986 (<i>n</i> = 7)	0.291	-0.264	-0.402	0.267	0.152	0.224
KYM 1987 (<i>n</i> = 14)	-0.040	0.147	-0.551 [*]	0.073	0.419	0.011
SAT (<i>n</i> = 9)	0.420	-0.164	-0.446	0.031	-0.314	-0.341
Stearic acid						
...						
ESA (<i>n</i> = 16)		0.239	-0.038	-0.409	-0.830 ^{***}	-0.390
KJL 1986 (<i>n</i> = 7)		-0.053	-0.112	-0.603	-0.544	0.013
KYM 1987 (<i>n</i> = 14)		0.017	-0.170	0.052	-0.019	0.491
SAT (<i>n</i> = 9)		0.264	-0.572	-0.516	-0.715 [*]	-0.246
Oleic acid						
...						
ESA (<i>n</i> = 16)			-0.911 ^{***}	-0.620 [*]	-0.260	-0.140
KJL 1986 (<i>n</i> = 7)			-0.755 [*]	-0.563	0.427	0.236
KYM 1987 (<i>n</i> = 14)			-0.884 ^{***}	-0.401	0.271	-0.464
SAT (<i>n</i> = 9)			-0.797 [*]	-0.832 ^{**}	-0.019	-0.207
Linoleic acid						
...						
ESA (<i>n</i> = 16)				0.349	0.072	0.107
KJL 1986 (<i>n</i> = 7)				0.310	-0.603	-0.545
KYM 1987 (<i>n</i> = 14)				0.278	-0.421	0.289
SAT (<i>n</i> = 9)				0.714 [*]	0.264	0.319
Linolenic acid						
...						
ESA (<i>n</i> = 16)					0.445	-0.048
KJL 1986 (<i>n</i> = 7)					0.271	0.119
KYM 1987 (<i>n</i> = 14)					0.225	0.010
SAT (<i>n</i> = 9)					0.220	0.587
Eicosenoic acid						
...						
ESA (<i>n</i> = 16)						0.379
KJL 1986 (<i>n</i> = 7)						0.552
KYM 1987 (<i>n</i> = 14)						0.440
SAT (<i>n</i> = 9)						-0.105

^aAbbreviations from Table I.

^bSignificance: ****P* < 0.001; ***P* < 0.01; **P* < 0.05.

1987).

The correlation of oleic and linolenic acids was significantly negative, and stearic and eicosenoic acids correlated negatively (Table VII). The associations between palmitic and linolenic and between linoleic and linolenic acid concentrations were positive. Linoleic and linolenic acid concentrations in oats have variously been found to be positively but insignificantly correlated (Forsberg et al 1974), significantly positively correlated (Saastamoinen 1987), and uncorrelated (Thro et al 1985). It was found that α - and γ -linolenic acids are synthesized by the desaturation of linoleic acid in plants (Mukherjee and Kiewitt 1987). The possible positive correlation between linoleic and linolenic acids can be explained by the synthesis pathway of these fatty acids. If the synthesis of linoleic acid is higher, a greater part of linoleic acid is desaturated to linolenic acid.

The negative correlations between stearic and eicosenoic acid concentrations can possibly be ascribed to the synthesis of very long-chain fatty acids (> C_{18}) from palmitic, stearic, and oleic acids by chain-specific elongases (Harwood 1988). Experiments with leek (*Allium porrum* L.) have shown that stearyl-CoA has been more effective as a precursor than palmitoyl-CoA in the presence of malonyl-CoA leading to the formation of fatty acids with a chain length of C_{20} - C_{26} (Lessire et al 1985). The significant negative correlations between the stearic and eicosenoic acid concentrations give the impression that eicosenoic acid is synthesized more effectively from stearic acid than from palmitic acid in oats.

Effects of Genetic and Environmental Factors on Fatty Acid Synthesis

In the oat material studied there seemed to be significant differences especially in oleic and linoleic acid concentrations, which were negatively correlated. However, environmental factors, especially low growth temperature, increase the synthesis of both oleic and linoleic acids. Genetic factors that increase linoleic acid concentration simultaneously decrease oleic acid content. Environmental factors that increase overall lipid synthesis

increase both linoleic and oleic acid concentrations. Genetic and environmental factors differ in their effects on the synthesis activity of different fatty acids.

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