OAT LIPIDS. I. COMPOSITION AND DISTRIBUTION OF LIPID COMPONENTS IN TWO OAT CULTIVARS[1]

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ABSTRACT

Groats from the oat cultivars Dal and Froker were ground and extracted first with ethyl ether to obtain free lipids (80%) and then with water-saturated butanol to obtain bound lipids (20%). Thin-layer chromatography and reflectance densitometry were used to separate and measure certain lipid components in groats (triglycerides, 1,2- and 1,3-diglycerides, free fatty acids, sterols, sterol glucosides, monogalactosyl monoglycerides, digalactosyl diglycerides, phosphatidyl choline, phosphatidyl ethanolamine, lysophosphatidyl choline, and lysophosphatidyl ethanolamine). Lipid components were also measured in four fractions from oat groats: bran, starchy endosperm, scutellum, and embryonic axis. The triglyceride component was most abundant. It averaged 41% of the lipids in groats, and 39 to 58% in the four groat fractions, with the scutellum and embryonic axis containing 50 and 58%, respectively. The next most abundant fraction was digalactosyl diglyceride; about 7% in the groats, and 8% in the endosperm and bran. Insufficient amounts were present in the embryo to measure. The other components, measured individually, each accounted for 6% or less of the total lipids. All components measured averaged 69% of the total lipids extracted from both cultivars and all fractions. Relative fatty acid composition of the free lipids extracted from four groat fractions and of the bound lipids extracted from bran and endosperm was measured by gas chromatography. Free lipids averaged more oleic and less palmitic acid (each about 7 percentage points) than bound lipids. Linoleic acid showed little change.

Lipid concentration in oats (*Avena sativa* L.) varies considerably. Brown *et al.* (1) reported a range of 3.8–8.5% in lipid concentration in 129 spring oat strains, and a range of 6.8–9.8% in 40 winter oat strains. Brown and Craddock (2) reported a range of 3.1–11.6% in oil concentration of oat groats among more than 4,000 entries in the world collection. Ninety per cent of the entries ranged from 5 to 9%. Lipid values reported in these papers were obtained through wide-line nuclear magnetic resonance. The potential for genetically changing the lipid concentration in oats appears good (3–5), and considerable variability of fatty acid composition among oat cultivars has also been reported (6,7). The feasibility of breeding oats for commercial oil and protein was discussed by Frey and Hammond (8). They reported that oats should contain at least 21% protein and 17% oil in order to make oats as profitable as soybeans under typical conditions in Iowa. Such a high oil concentration, if obtainable, would undoubtedly alter the use of oat groats for food as we now know it. Currently, little work is being done in the U.S. to deliberately change oil concentration in oats.

Since lipids are the most concentrated form of energy in food, amounts present in oats could represent an important energy contribution to food and feed, particularly as a feed for animals such as dairy cattle. Also, Chancellor and Goss

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(9) recently stated that in the future, food caloric production is expected to be more critical than protein production. One reason given was that recent evaluations of protein requirements for humans have shown a lower average need than previously believed necessary, pregnant women and children excluded.

Little work has been done on the identification of components in oat lipids. In 1962, Aylward and Showler (10) isolated a glyceride fraction and two purified phosphatide fractions. Recently, Price and Parsons (11) used thin-layer chromatography to identify several oat lipid components. They also reported the relative amounts of neutral lipid, glycolipid, and phospholipid in oats. In this paper, we report on the lipid concentration and distribution of several lipid components in two oat cultivars, and in four oat groat fractions.

MATERIALS AND METHODS

Fatty acid and lipid concentrations were determined on two oat cultivars: Dal, with high lipid concentration, and Froker, with medium lipid concentration, each grown in two crop years (1973–74) at one location (Wisconsin). These are the two leading oat cultivars grown in Wisconsin. Seed samples from three field replications for each cultivar in each year were hand-dehulled to produce groats. A portion of these groats from each of the three field replicates was used for lipid determination. The embryonic axis and scutellum were removed from a second sample of these groats (only two of the three field replicates) as described earlier (12), and these degermed groats were milled in a Brabender Quadrumat Jr.® flour mill to produce bran and starchy endosperm. Subsequently, fatty acid determinations were performed on lipids from oat groats and groat fractions.

Lipid Extraction

Lipids from ground oat groats and the groat fractions were extracted with diethyl ether (ether) for 7 hr on a Goldfisch extractor, evaporated to dryness under vacuum on a rotoevaporator at 50° C, weighed, and the weight recorded as free lipids. The ether-extracted samples were extracted again with water saturated n-butanol (WSB) for 0.5 hr, and evaporated to dryness under vacuum. This mixture was taken up in chloroform, rotoevaporated, and the lipids were weighed and recorded as bound lipids. All results were reported on a dry basis. Three determinations were made on each sample when measuring fatty acids of ether-extracted lipids. Two determinations were made on each sample for WSB lipids.

Thin-Layer Chromatography

Silica gel thin-layer chromatography was used to separate and quantitate 12 lipid components from each sample. They were triglycerides (TG), free fatty acids (FFA), 1,2-diglycerides (1,2-DG), 1,3-diglycerides (1,3-DG), sterols, sterol glucosides (SG), phosphatidyl ethanolamine (PE), monogalactosyl monoglycerides (MGMG) (tentatively identified), digalactosyl diglycerides (DGDG), phosphatidyl choline (PC), lysophosphatidyl ethanolamine (LPE), and lysophosphatidyl choline (LPC). Attempts to quantitate the sterol esters were unsuccessful because the amounts present were too low. Other spots also appeared which were not identified or measured. Spot identification on the

plates was made by comparing R_f values with 11 standards (no standard for MGMG), and by color reactions specific for certain compounds after treatment with specific reagents (13). For quantitation, exact amounts of the lipids were spotted on "Safety-Kotes" (Applied Science, State College, Pa.). The thin-layer plates were developed in selected solvent systems, heated at 180°C for 20 min, and the spots that resulted were measured by reflectance densitometry with a Kontes densitometer (Vineland, N.J.). Exact amounts of standards were included on every plate, and care was taken to regulate spot size and density. The relation between spot weight and spot density was linear between 20 μg/spot, the minimum that could be measured, and 100 μg/spot. Spots not identified were not measured, but their combined values were estimated by subtracting the sum of the lipid component weights measured from the weight of lipid spotted. These solvent systems were used to develop the thin-layer plates: sterol esters, 100% carbon tetrachloride; TG and FFA, petroleum ether, ethyl ether, acetic acid, 90:15:1.5; 1,3-DG, 1,2-DG, and free sterols, same solvents, 60:50:1.5; and the remaining lipid components, chloroform, methanol, water, 65:15:3. Multiple development (six times) was used to separate the last group. All solvent relations are v/v/v.

Limited, further analysis was done to identify components of the free sterols. Thick-layer (0.50-mm Silica Gel G) plates were streaked with oat lipids and developed in appropriate solvent systems to obtain sufficient quantities of free sterols for further analysis by gas-liquid chromatography (glc). The sterol band was visualized by exposing the plate briefly to iodine vapor. The band was scraped from the plate, and the sterols eluted with ethyl ether and saved for analysis by glc.

TABLE I
Lipid Concentration in the Groats and Oat Fractions of Two Oat Cultivars

Source	Ether Extraction[a] (Free Lipids) % ± SD		WSB Extraction[a] (Bound Lipids) % ± SD	
Dal				
Groats	8.0	0.75	1.6	0.22
Hulls	2.3	0.71	0.6	0.06
Bran	9.5	0.21	1.2	0.14
Endosperm	6.8	0.25	1.0	0.11
Scutellum	20.6	2.04	2.8	0.80
Embryonic axis	12.6	1.63	3.3	1.00
Froker				
Groats	5.5	0.30	1.4	0.35
Hulls	2.0	0.11	0.6	0.06
Bran	6.4	1.41	1.3	0.14
Endosperm	5.2	0.35	1.0	0.21
Scutellum	20.4	0.91	4.2	0.37
Embryonic axis	10.6	0.61	4.1	0.63

[a]Dry basis.

TABLE II
Analysis of Variance of Lipid Constituents in Oat Groats and Groat Fractions

Source	df	Mean Squares[a]												
		TG	FFA × 100	1,2-DG × 100	1,3-DG × 100	Sterols × 100	SG × 100	PE × 100	MGMG[b] × 100	DGDG[b] × 100	PC × 100	LPE[b] × 100	LPC[b] × 100	Others
Oat Groats														
Year (Y)	1	5.17	873.13**	6.94**	9.03**	34.31**	0.06	65.15**	0.57	1.12	118.64**	81.03**	16.45**	53.89*
Error a	4	3.01	0.41	0.04	0.06	0.03	0.01	0.02	0.09	0.30	0.17	0.10	0.26	4.16
Cultivars (C)	1	423.64	4.71**	2.22	0.01	5.03	2.49	0.12	0.12	1.05	0.01	0.17	0.49	372.63
Y × C	1	129.75**	0.42	1.33**	0.01	0.24**	2.86**	0.02	0.06	0.01	0.04	0.01	0.42	124.87**
Error b[c]	4	0.43	0.08	0.02	0.02	0.01	0.05	0.04	0.30	0.86	0.34	0.05	0.23	4.40
Oat Groat Fractions														
Year (Y)	1	46.7	1.16	11.27	0.59	49.61*	252.67	24.50	...	...	28.78	...	...	1.46
Error a	2	27.4	2.74	21.39	3.60	1.81	23.83	6.89	...	...	4.80	...	...	0.43
Cultivars (C)	1	384.5	3.35	2.76	0.87	0.01	5.40	23.36	...	...	8.85	...	...	1.78
C × Y	1	0.7	2.16	1.81	13.67	2.46	23.09	19.59	...	...	28.14	...	...	0.04
Error b	2	72.1	2.60	3.26	5.14	1.11	27.90	5.57	...	...	5.80	...	...	0.46
Fraction (F)	3	612.5**	15.75*	42.45**	5.81**	0.71	19.40**	62.58**	...	...	16.02*	...	...	1.56**
F × Y	3	28.7	6.10	3.05	0.51	0.21	2.68**	2.24	...	...	7.20	...	...	0.35
F × C	3	35.9	5.23	2.62	3.63	0.33	0.80	2.65	...	...	39.72**	...	...	0.62*
F × Y × C	3	65.6	16.23*	1.12	0.43	0.26	0.98	2.45	...	...	30.66**	...	...	0.18
Error c	12	16.1	3.00	1.03	0.80	0.76	0.41	3.66	...	...	3.50	...	...	0.14

[a]All values, except triglycerides (TG) and groat "others," are from transformed data ($\sqrt{x + 1/2}$).
[b]No determinations made in embryonic axis and scutellum.
[c]Years were random and cultivars were fixed effects; therefore, when % × C interaction was significant, it was used to test cultivars.

TABLE III
Concentration of Lipid Components in the Total Lipids of Oat Groats and Groat Fractions[a]

Source	Lipid Component (% Total)												
	TG	FFA	1, 2-DG	1, 3-DG	Sterols	SG	PE	MGMG	DGDG	PC	LPE	LPC	Others[b]
Oat Groats													
1973													
Dal	50.6a	11.0a	1.3a	1.7a	0.5c	0.9b	0.8b	4.0a	6.9a	3.2b	0.4b	2.7a	16.0d
Froker	32.4d	10.0a	0.9ab	1.7a	0.9b	1.4a	0.8b	4.1a	7.3a	3.2b	0.4b	2.7a	34.2a
1974													
Dal	42.9b	2.3b	0.7ab	1.2a	1.4a	1.1b	2.2a	4.2a	7.3a	6.0a	1.7a	1.8b	27.2b
Froker	37.5c	2.0b	0.6b	1.2a	1.7a	1.1b	2.2a	4.3a	7.6a	6.1a	1.7a	2.1b	31.9c
Groat Fractions[c]													
Bran	38.6c	2.8a	1.1b	1.9a	0.9a	0.9b	2.5a	4.0a	8.0a	3.6a	1.7a	2.9a	31.0b
Endosperm	41.0c	3.0a	1.1b	1.8a	0.9a	1.2a	2.3a	4.5b	8.0a	3.5a	1.7a	3.0a	28.0b
Scutellum	50.1b	2.1b	2.1a	1.3b	0.7a	0.5c	0.9b	...	...	2.6b	...	...	39.7a
E. axis	57.7a	2.0b	2.4a	1.6a	0.8a	0.8b	1.1b	...	...	2.8b	...	...	30.8b

[a]Values followed by the same letter within each lipid component, within groats, and within fractions are not significantly different from each other, protected LSD test, at the 5% level.
[b]Lipids not measured, values obtained by difference.
[c]Averages across years and cultivars. MGMG, DGDG, LPE, and LPC were not measured in the scutellum or embryonic axis.

Gas-Liquid Chromatography

The gas chromatograph was equipped with a flame ionization detector, and nitrogen was used as a carrier gas. Fatty acids were converted to methyl esters for glc analysis by the method of Metcalfe *et al.* (14). Lipids were hydrolyzed with 0.5*N* sodium hydroxide and treated with 14% boron trifluoride in methanol. Separation of the fatty acid methyl esters was accomplished in a 3.17 mm (o.d.) × 2.54-m stainless-steel column, packed with 20% diethylene glycol succinate on 80/100 mesh Chromosorb WAW (Supelco, Inc., Bellefonte, Pa.). Column temperature was 190° C. Trimethylsilyl ether derivatives (prepared with Sil-Prep, Applied Science Labs., Inc.) of the free sterols obtained from thick silica gel plates were separated in a 3.17 mm (o.d.) × 2.54-m stainless-steel column packed with 3% SE-30 on Gas-Chrom Q (Applied Science Labs., Inc., State College, Pa.). Column temperature was 265° C.

RESULTS AND DISCUSSION

Concentrations of lipids extracted from oat hulls, groats, and four groat fractions are shown in Table I. Results shown are an average of three replications for two years for the groats and hulls, and two replications for 2 years for the remainder. The cultivar Dal contained a higher concentration of lipid than Froker. Groats from the cultivars contained about 80% free lipids (ether extract), and 20% bound lipids (WSB extract). Hulls had the smallest lipid concentration, and the embryo fractions (embryonic axis and scutellum) the greatest. Most of the total lipid found in the oat kernel was in the bran and starchy endosperm, because these kernel fractions comprise the greatest part of the total kernel weight (12).

Analyses of variance for the 12 lipid components are shown in Table II. In oat groats, FFA, DG, sterols, PE, PC, LPE, and LPC showed highly significant differences between years. FFA was the only lipid component to express a significant difference between cultivars. Performances of cultivars were dependent on year grown for TG, 1,2-DG, sterols, and SG as evidenced by significant year-by-cultivar interaction. Significant differences in oat groat fractions occurred in all lipid constituents except sterols.

Table III shows the concentration (% of total lipids) of lipid components in oat groats of each cultivar within each year and groat fractions across cultivars (cultivar by fraction not significant). Significant differences within each lipid component between oat cultivars in each year and among groat fractions also are shown, and they are based on protected least significant difference (LSD) values, as discussed by Federer (15). In oat groats, the TG component was largest, and Dal contained more than Froker: 46.8 and 35.0% (averages), respectively. These performances varied by year also. Similarly, TG dominated each of the four groat fractions. The embryonic axis contained the greatest concentration, scutellum next, followed by the endosperm and bran. DGDG was the second most abundant lipid component measured, but it only accounted for about 7% of the lipids in oat groats, and 8% of those in the bran and endosperm. Insufficient amounts were present in the embryo to measure. Other components, measured individually, accounted for 6% or less of the total lipids. It is of interest that amounts of DGDG were too small to be measured in the embryo fractions, although PC and PE could be measured.

TABLE IV
Analysis of Variance of Fatty Acids in Oat Groats and Groat Fractions of Two Cultivars

Source of Variation	df	Mean Squares					
		Myristic[a]	Palmitic	Stearic[a]	Oleic	Linoleic	Linolenic[a]
Ether Extract (Free Lipids)							
Years (Y)	1	0.003	9.44**	0.458	17.85	91.56	0.003
Error a	2	0.709	0.01	0.242	11.21	13.78	0.190
Cultivars (C)	1	2.004	236.32**	0.081	120.71	11.71	2.032
C × Y	1	0.001	2.72	0.029	38.19	23.14	0.039
Error b	2	0.462	0.30	0.347	25.14	50.30	0.543
Fractions (F)[b]	4	0.135	59.15**	0.517**	431.42**	64.68**	2.721**
F × Y	4	0.049	2.05	0.118	8.14	6.00	0.099
F × C	4	0.671	22.77**	0.205*	22.12*	14.74	0.108
F × C × Y	4	0.010	2.76	0.054	4.37	8.84	0.305
Exp. error[c]	16	0.307	3.19	0.051	6.02	5.10	0.134
Sampling error	80	0.010	0.92	0.025	0.64	0.46	0.021
WSB Extract (Bound Lipids)							
Years (Y)	1	0.001	82.60	0.013	412.66*	100.49	0.362
Error a	2	0.189	10.69	0.381	18.97	13.28	0.020
Cultivars (C)	1	0.735	118.89*	0.466	205.20	33.65	0.548
C × Y	1	0.001	17.88	0.010	4.32	3.49	0.021
Error b	2	0.034	5.98	0.382	26.62	23.06	0.532
Fractions (F)[b]	2	1.522*	56.38	0.388	90.22	38.24	0.067
F × Y	2	0.097	39.50	0.427	81.54	14.97	0.010
F × C	2	0.099	64.34	0.241	124.88	13.74	0.226
F × C × Y	2	0.022	27.17	0.260	21.20	0.42	0.219
Exp. error[c]	8	0.293	24.80	0.392	32.37	15.22	0.086
Sampling error	48	0.028	1.28	0.103	0.30	0.76	0.028

[a]Values from transformed data ($\sqrt{\times + 1/2}$).
[b]Fractions include groat, bran, and endosperm for both extractions, and scutellum and embryonic axis for ether extract.
[c]Experimental error was significantly larger than sampling error in all cases.

In Table III, the difference in FFA concentrations between 1973 and 1974 was undoubtedly due to extraction procedure. The 1973 groat samples were ground one day and extracted the next, while the 1974 samples were ground and extracted immediately. Lipase, which is very active after a sample is ground, caused considerably more FFA to be released in the 1973 samples than in any of the others. These data indicate that large amounts of FFA do not naturally exist in mature oat groats.

Free sterols were collected on thick silica gel plates in sufficient quantities for

TABLE V
Concentration (%) of Fatty Acids in Free and Bound Lipids Extracted from Oat Groats and Fractions

Fraction-Cultivar		Fatty Acid					
		Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Ether Extract (Free Lipids)[a]							
Groats	Dal	0.4	17.0*	2.6	41.7	36.9	1.4
	Froker	0.5	20.6	1.9*	36.9*	38.8	1.3
	Mean	0.4a	18.8b	2.2a	39.4a	37.9c	1.3c
Bran	Dal	0.3	15.7*	1.5*	39.7	40.7	2.1
	Froker	0.5	20.5	2.4	37.0*	38.4	1.2*
	Mean	0.4a	18.1b	1.9a	38.4ab	39.6b	1.6c
Endosperm	Dal	0.6	16.9*	2.0	38.5	39.9	2.0
	Froker	0.7	21.0	2.5	36.2*	38.7	0.9*
	Mean	0.6a	18.9b	2.3a	37.4b	39.4b	1.4c
Scutellum	Dal	0.2*	20.9	1.1	34.6	39.8	3.3
	Froker	0.8	21.4	1.2	34.5	39.6	2.5
	Mean	0.6a	21.1a	1.2b	34.5c	39.7b	2.8b
Embryonic axis	Dal	0.2*	21.2	1.9	28.9	43.0	4.8
	Froker	1.8	22.1	1.9	28.8	42.0	3.4*
	Mean	0.9a	21.6a	1.9a	28.8d	42.5a	4.1a
WSB Extract (Bound Lipids)[b]							
Groat		0.9b	25.7a	2.0a	28.8a	41.0a	1.4a
Bran		1.6a	25.7a	1.6a	27.2a	42.2a	1.4a
Endosperm		0.6b	27.3a	2.4a	28.4a	39.7a	1.3a

[a]Means between fractions within a fatty acid followed by same letter not significantly different from each other at the 5% level—protected LSD.
[b]Interaction between fraction and cultivar not significant, therefore mean across cultivars presented.

glc analysis. Only single replicates of each cultivar from the 1973 crop year were analyzed. Standards were used to identify all peaks except Δ^5 avenasterol; this was tentatively identified by comparing relative retention time with data from Knights (16). Little difference was noted between the two cultivars. Average values of the sterols measured were cholesterol, 2%; brassicasterol, 4%; campesterol, 10%; stigmasterol, 8%; sitosterol, 69%; and Δ^5 avenasterol, 7%. The value of sitosterol, the major sterol, was higher than that reported earlier by Knights (17), although he also reported values for three additional sterols that we did not.

Analyses of variance for fatty acids in the free and bound lipids of oat groats and groat fractions are shown in Table IV. In the free lipids only, palmitic acid was significantly influenced by the cultivar or year in which it was grown. Differences ($P \leqslant 0.01$) occurred among fractions in all fatty acids from these lipids except myristic. The significant interaction of fractions by cultivars for palmitic, stearic, and oleic acid indicates that the distribution of these fatty acids within the fractions is dependent upon the cultivar. The fatty acids in the bound lipids were very stable between years, cultivars, and fractions.

Table V shows the fatty acid composition of each fraction (groats included as a fraction) from each oat cultivar in the free lipids, and fractions, expressed as an average of both cultivars in the bound lipids. Palmitic, oleic, and linoleic acid are the major fatty acids. In the bound lipids, only myristic acid was found to be disproportionally distributed between the bran (1.6%) and endosperm (0.6%). In the free lipids, in general, the bran and endosperm had comparable concentrations of fatty acids, as did the whole groats. The scutellum and embryonic axis contained less oleic acid, and more palmitic, linoleic, and linolenic acid than did the whole groat. All parts contained similar concentrations of myristic acid, but the scutellum had less stearic acid. Oleic acid showed the greatest range in concentration of the fatty acids in the groat parts; 38.4% in the bran to 28.8% in the embryonic axis.

It is of interest that the cultivars differed in concentrations of fatty acids, depending upon the source of the fatty acid determination. For example, the cultivar Dal had significantly less palmitic and more oleic acid in the groats, bran, and endosperm than did Froker. This would imply that at least for palmitic and oleic acid, some genetic variability does exist such that alteration of these acids could be achieved through appropriate breeding procedures, which is in agreement with a previous study (7).

Oat lipids apparently are similar in composition to lipids from other cereal crops, with the exception that oat groats generally contain a higher concentration of lipid within the kernel. This higher concentration is important as an energy source in feeds and, if the future demands it (9), oat lipids also may be important as an energy source in foods.

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