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Analysis of Oil Composition in Cultivars and Wild Species of Oat (*Avena* sp.)

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Oil quality and content were analyzed in 33 accessions from 13 wild species and 10 accessions of cultivated oat. Wild oat species tended to have higher oil and 18:1 fatty acid (FA) contents and lower amounts of 18:2 and 18:3 FAs as compared to cultivated oats. In addition to common FAs, minor amounts of several hydroxy and epoxy FAs were also present in the oat oil and mainly confined to specific lipid classes. These unusual FAs included the previously reported 15-hydroxy $18:2^{\Delta 9,12}$ (avenoleic acid) mostly found among polar lipids and a novel 7-hydroxyhexadecanoic acid located to 1,2-diacylglycerol. The present study highlights the potential of making use of the existing germplasm, consisting of wild oat species, in breeding programs for achieving new oat varieties that produce a range of oils with different FA compositions as well as having high oil contents. However, in one matter, oats apparently lack genetic diversity and that is for oil qualities that are highly enriched in the omega 3 (ω-3) FA 18:3. Consequently, developing oat cultivars with highly unsaturated oils will need involvement of other techniques such as biotechnology.

KEYWORDS: ***Avena*** **spp.; groat; oil; lipid classes; fatty acid; epoxy; hydroxy**

INTRODUCTION

Oat (*Avena sativa*) is a well-known cereal crop that is mainly used as feed. A small but important portion of the crop is also used for other purposes such as a source for pharmaceutical products, biomaterials and energy, and food (*1*−*3*). However, there is expansion potential in the latter since the nutritional benefits using oat as a food source recently have been recognized (*3*). Oat has a high content of soluble fibers, that is, β-glucans, which have unique health-promoting properties, a favorable amino acid profile, high levels of antioxidants, and are rich in important vitamins and minerals (*3, 4*).

As compared to other cereals apart from maize, oat has a high capacity to accumulate substantial amounts of oil in its grains. While commercial cultivars can accumulate up to 10% oil, contents of up to 18% have been achieved after using recurrent phenotypic selection for high groat oil content (*5*). A high content of oil that is rich in both unsaturated and saturated fatty acids (FAs) provides both nutritional and economical profits in oat production. The high level of antioxidants is furthermore an important factor to the high stability of oat oil (*6*).

Recently, there has been a growing interest in nutritional FAs in our society. This comes from increasing awareness of the importance of very long and polyunsaturated FAs such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and arachidonic (AA) acids for the prevention of pathologies such as heart disease, arthritis, and inflammatory and autoimmune diseases (*7*). The unsaturated 18:2 (ω-6) and 18:3 (ω-3) plant FAs have here a significant role as precursors in the synthesis of very long polyunsaturated moieties in humans. It has been recognized that an unbalanced ω-6/ω-3 FA ratio in the western diet might be a factor behind many modern health problems. The relative proportion of ω-6 to ω-3 FAs in food rather than high intake of the long-chain ω-3 FAs alone has been suggested to be of importance, with a ratio of 1−4 as most favorable (*8, 9*). While cultivated oat has a high level of ω-6 FA (36−47% 18:2), the level of ω-3 FA is very low (1−2% α-18:3) (*10, 11*). Increasing the level of 18:3 FA in oat would therefore improve an important nutritional parameter.

Improved oat cultivars with better performance in groat components can be achieved by utilizing wild species in a breeding program. Interspecific hybridization has been documented as an efficient source for improving oat grain quality and obtaining high-yielding lines (*10, 12*). Sources for traits such as high protein and oil amount, disease resistance, and beneficial agronomic traits can be found among wild oats of all ploidy levels (*13*). However, to our knowledge, available data on FA composition of oat grains are mostly based on the

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Table 1. Oil (Percent of Dry Weight Seed) and Lipid Content (Percentage of Total Lipids) in Dehulled Grains of Different Species (2*n*, 4*n*, and 6*n*) of Wild Oat ($n = 3$)[a]

species[b]	oil content	PL	1,2-DAG	1,3-DAG	unknown lipid	FFA	TAG1	TAG2	TAG
A. clauda	8.7	16.6	3.1	4.4	1.6	3.2	1.4	0.8	68.8
A. longiglumis	7.9	19.0	1.9	1.0	1.2	1.7	1.6	1.1	72.2
A. canariensis 1	8.6	10.8	1.7	4.2	1.8	0.9	1.4	1.2	77.6
A. canariensis 2	9.4	22.7	1.5	2.1	1.8	2.1	0.6	0.8	68.0
A. hirtula 1	8.3	12.8	1.2	1.7	1.3	1.4	1.3	0.9	79.0
A. hirtula 2	8.7	14.0	1.2	1.8	0.9	1.6	0.9	0.8	78.5
A. hirtula 3	8.7	11.8	1.3	1.4	1.3	1.8	0.6	0.5	81.3
A. wiestii 1	8.4	12.9	1.6	2.2	1.5	1.1	1.4	1.2	77.9
A. wiestii 2	8.0	13.7	1.4	1.7	1.7	3.0	1.7	1.2	75.2
A. barbata 1	6.9	16.0	1.4	2.6	1.1	3.7	0.7	0.7	73.7
A. barbata 2	8.2	15.5	1.2	2.2	1.2	1.8	0.8	0.6	76.6
A. barbata 3	7.7	19.3	1.2	1.8	0.7	1.8	1.1	0.8	73.1
A. barbata 4	7.0	16.5	1.0	1.5	1.1	1.6	1.0	1.0	76.2
A. barbata 5	8.5	16.9	2.3	3.1	2.1	1.5	0.7	0.6	72.6
A. vaviloviana 1	6.6	19.2	1.9	2.3	1.6	2.3	1.5	1.4	69.4
A. vaviloviana 2	7.2	17.0	2.6	2.1	1.5	1.5	1.4	1.2	72.6
A. vaviloviana 3	7.6	16.1	1.1	2.2	1.1	1.1	1.0	1.0	76.1
A. magna 1	6.3	15.3	1.1	1.8	1.7	1.2	2.1	1.2	75.6
A. magna 2	7.6	11.9	0.9	1.5	0.9	1.4	1.1	0.9	81.3
A. magna 3	7.2	14.1	0.9	2.0	1.2	1.0	1.6	1.2	77.8
A. magna 4	5.2	23.0	1.3	2.5	1.2	1.5	1.2	1.1	68.2
A. murphyi 1	8.8	16.8	1.6	3.0	1.2	0.7	0.8	0.7	74.8
A. murphyi 2	6.6	18.0	1.7	2.8	1.1	1.2	1.1	0.9	72.9
A. fatua 1	9.2	13.2	1.5	1.9	1.1	1.6	1.2	0.9	78.5
A. fatua 2	6.6	15.2	1.4	1.9	1.1	0.2	1.4	1.0	76.7
A. fatua 3	6.7	12.6	0.9	1.8	1.4	1.6	1.2	0.7	79.7
A. ludoviciana 1	8.4	12.8	2.1	2.1	1.2	0.9	1.0	1.1	78.5
A. ludoviciana 2	8.5	19.0	1.1	2.0	1.1	1.4	1.0	1.0	73.3
A. ludoviciana 3	8.5	13.0	1.0	1.7	0.8	2.7	0.8	0.6	79.4
A. ludoviciana 4	8.7	17.7	1.6	2.5	0.8	0.5	0.8	0.3	75.7
A. sterilis 1	7.4	21.0	1.1	1.9	1.1	2.8	1.0	0.9	70.2
A. sterilis 2	7.3	11.8	1.1	1.6	0.8	1.5	1.6	0.9	80.4
A. sterilis 3	6.9	18.0	0.9	1.8	0.8	1.7	1.0	0.8	74.6
mean	7.8	15.9	1.5	2.2	1.2	1.6	1.2	0.9	75.4
SEM[c]	0.1	0.4	0.1	0.1	0.1	0.1	0.1	0.0	0.5
LSD (0.05)[d]	0.9	3.5	1.0	1.0	0.7	1.3	0.7	0.5	5.8
F value	8.8***	7.3***	1.9*	3.5***	1.9*	2.6***	2.1**	1.7*	3.3***

[a] Abbreviations: PL, polar lipids; 1,2-DAG, 1,2-diacylglycerol; 1,3-DAG, 1,3-diacylglycerol; FFA, free FAs. *F* values are from one-way ANOVA. ***Significant at $p < 0.001$; **significant at $p < 0.01$; and *significant at $p < 0.05$. [b] Numbers indicate different accessions of the same species taken for the analysis. [c] SEM, standard error of mean. [d] Fisher's protected LSD.

investigation of cultivated and wild growing oats with one ploidy level, $2n = 42$. Most often, *Avena sterilis* has been the focus of investigations as one of the closest relatives to the most prevalent cultivated species *A. sativa* (*13*). However, because the *Avena* genus consists of 31 known species, four cultivated and 27 wild-growing (*13, 14*), there is reason to investigate a broader selection of wild species for traits with relevance to oil quality and FA composition, which can be used in conventional breeding.

This study was therefore undertaken to give detailed information on the oat groat—oil content, FA composition, lipid classes' compositions, and their FA compositions of 13 oat species—including both cultivated and wild-growing oats. Emphasis was specifically on accessions with traits useful in elevating the level of 18:3 FA.

MATERIAL AND METHODS

Oat Material. The oat material was supplied as seeds of 10 cultivated and 33 wild-growing accessions from N. I. Vavilov All Russian Institute of Plant Industry world collection. Wild-growing oat accessions included 12 species of different geographical origin with all three ploidy levels represented ($2n = 2x = 14$, $2n = 4x = 28$, and $2n = 6x = 42$), and cultivars were all hexaploid *A. sativa* (Supporting Information, Tables 1 and 2). All samples were reproduced at Pavlovsk experimental station, Saint-Petersburg, Russia, which is situated 30 km from Saint-Petersburg in the latitude 59°44′ North and longitude 30° 24′ West. The cultivation area was located amidst the North-Western part of the not black-earth zone of Russia having a semisandy cespitose podzol soil, with a humus content of 2.2%, a content of P_2O_5 and K_2O of 5.92 and 132 mg/100 g, respectively, and a pH of 6.3. This area received sufficient humidity with a sum of precipitation during the vegetative period of 347 mm. The vegetative period with stable temperature higher than 5 °C started in May and ended at the end of September.

The material was planted May 23 and harvested, depending on maturity, at the end of August. Plants were treated against frit fly (June 11) and weeded when necessary during the cultivation.

Chemicals. All chemicals, solvents, lipid references, and FAs standards and references were reagent grade and purchased from Merck (Darmstadt, Germany), Sigma (St. Louis, MO), or Larodan Fine Chemicals (Malmö, Sweden).

Oil Content. Oat samples were dehulled manually before analysis. To define the oil content, three seeds per sample were crushed and directly treated with 2 mL of methanolic H_2SO_4 (2% v/v) for 80 min at 90 °C. Internal standard (heptadecanoic acid methyl ester) was added, and FA methyl esters were extracted with hexane. The hexane phase was used for gas chromatography (GC) analyses. The oil content was calculated by adding areas of individual FAs methyl esters after separation on a GC and using the formula:

$$\text{oil (\%)} = \frac{W_{is} \Sigma A_{fa}}{A_{is} W_s} \times 100$$

where "*W*" is the weight, "*A*" is the area, "is" is the internal standard, "fa" is the FA methyl ester, and "s" is seeds. It should be noted that

the oil content as analyzed in this study was based on the FA content in the oil. Lipids not containing FAs such as free sterols were therefore not included.

Lipid Classes Content. To analyze the contents of the lipid classes and their FA compositions, oil was extracted according to Bligh and Dyer (*15*) as follows: three seeds per sample were homogenized in 3.75 mL of MeOH−$CHCl_3$ (2:1, v/v) with the addition of 1 mL of 0.15 M acetic acid in a Potter−Elvehjem glass homogenizer. The extract was transferred to a screw-capped tube, and the homogenizer was rinsed with 1.25 mL of chloroform that was added to the extract. Water (1.25 mL) was added to the extract that was mixed by vortexing and phase separated in a centrifuge. The lipid-containing chloroform phase was transferred to another tube and evaporated to dryness under N_2. The residue was dissolved in 35 μL of chloroform and applied on a silica gel thin-layer chromatography plate (TLC) (Silica 60; Merck, Darmstadt, Germany). The lipids were separated by developing the plate with hexane/diethyl ether/acetic acid (35:15:0.01, v/v/v.). After the TLC plate was stained with primulin, spots corresponding to different lipid classes were identified under UV light by comparing them with authentic lipid standards.

FA Composition of Lipid Classes. The TLC plate was moistened with water, and individual spots of gel were removed into a glass tube. Dry MeOH was added to the gel sample and evaporated by flushing with N_2 to remove traces of water. The sample was then methylated in situ with 2 mL of methanolic H_2SO_4 (2% v/v) for 40 min at 90 °C. Internal standard, heptadecanoic acid methyl ester, was added, and the FA methyl esters were extracted in hexane. The dried hexane phase was dissolved in hexane and analyzed on a GC. Three replicates for each analysis were carried out.

GC. Analyses of FAs were carried out on a Shimadzu-GC 17A (Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column coated with CP-wax 58 CB (Chrompack UK Ltd.) and a flame ionization detector. Helium was used as the carrier gas. An initial column temperature of 160 °C was increased to 250 °C at a rate of 3 °C/min and maintained until the completion of the analysis. Injection and detector temperatures were 230 and 270 °C, respectively. Two microliters of the hexane extract was injected, and the FA methyl esters were identified by comparison of retention times with those of standard samples containing a range of FA including all common and unusual FAs in this study. Peak integration was performed using the Shimadzu CLASS VP Version 4.3 (Shimadzu, Kyoto, Japan) chromatography software with the peak area of the internal standard as reference.

The percentage of each lipid fraction (*lf*) was calculated by the formula:

$$\mathrm{lf}\ (\%) = \frac{\sum N_{\mathrm{fa}}}{\sum N_{\mathrm{FA}}} \times 100$$

where N_{fa} is the amount of FA (nmol) in one lipid fraction and N_{FA} is the amount of FA (nmol) in all lipid fractions of a certain sample.

Isolation and Characterization of 7-Hydroxyhexadecanoic Acid from Cereal Seeds. Ground cereal seeds (100 g) were extracted with $CHCl_3$−MeOH (2:1) as previously described (*16*). The resulting oil was treated with 0.4 M NaOH in ethanol−water (4:1, v/v) at 70 °C for 1 h. Material extracted with diethyl ether was subjected to open column SiO_2 chromatography. Elution with diethyl ether/hexane (1:1, v/v) afforded a fraction of mixed oxygenated FAs, which was methyl-esterified and rechromatographed on a second column of SiO_2. Elution with diethyl ether/hexane (2:8, v/v) afforded a hydroxyester fraction, which mainly constituted of isomeric methyl hydroxyoctadecadienoates but also contained a new shorter chain hydroxyester. This compound was obtained in pure form following reversed-phase high-performance liquid chromatography (HPLC) using a column of Nucleosil 100-7 C_{18} (250 mm × 10 mm) and a solvent system of acetonitrile/water (6:4, v/v) (elution volume, 257 mL) followed by TLC using a solvent system of ethyl acetate/hexane (25:75, v/v) (R_f 0.42). Crystallization from hexane yielded pure methyl 7-hydroxyhexadecanoate (mp 44−45 °C). Part of this material was saponified and crystallized from ethyl acetate−hexane affording 7-hydroxyhexadecanoic acid having a mp of 62−63 °C. An aliquot of crystalline 7-hydroxyhexadecanoic acid was treated with diazomethane followed by trimethylchlorosilane/hexamethyldisilazane/pyridine (2:1:2, v/v/v) to afford the methyl ester-trimethylsilyl ether derivative, which was analyzed by GC-MS.

Estimation of 7-Hydroxyhexadecanoic Acid in Different Cereal Seeds. Samples of 1.0 g of ground seeds of wheat (cv. Dragon and Kosack), oat (cv. Freja and Vital), rye (cv. Motto and Marder), and barley (cv. Frost and Kinnan) were homogenized in 50 mL of $CHCl_3$−MeOH (2:1, v/v) containing methyl 17-hydroxystearate (122 μg) as an internal standard. The extracted materials were saponified, purified by preparative TLC as methyl esters, and subjected to GC analysis. The peak areas of methyl 7-hydroxyhexadecanoate (eluting at 7.2 min) and the internal standard (eluting at 15.2 min) were integrated, and the amounts of 7-hydroxyhexadecanoic acid were calculated using a standard curve.

Absolute Configuration of 7-Hydroxyhexadecanoic Acid. The configuration at C-7 of the new hydroxy acid was determined by stepwise degradation of the carbon chain and steric analysis of the resulting β-hydroxy acid as previously described (*17*). Thus, 7-hydroxyhexadecanoic acid (5.5 mg) was acetylated and subsequently refluxed with $KMnO_4$ in acetone for 7 h. The product was treated with diazomethane and deacetylated by treatment with sodium methoxide in methanol. From the mixture of homologues, methyl 3-hydroxydodecanoate was isolated by preparative TLC using a solvent system of ethyl acetate/hexane (3:7, v/v). The β-hydroxyester was derivatized with 2(*S*)-phenylpropionyl chloride, and the resulting ester was analyzed by GC using the 2(*S*)-phenylpropionyl derivative of methyl 3(*R*,*S*)-hydroxydodecanoate as a reference.

Identification of Minor Oxygenated FAs in Seed Oils from Oat. Seed oils were fractionated by TLC, and silica gel corresponding to the different lipid classes was scraped off and eluted with ethanol (3 mL) containing butylated hydroxytoluene antioxidant (125 μg). Sodium hydroxide (2 M; 0.6 mL) was added, and the samples were purged with argon and kept at 50 °C for 1 h. The solutions were acidified to pH 3, extracted with diethyl ether, and methyl-esterified by treatment with diazomethane. The resulting samples were analyzed by GC-MS before and after additional treatment with trimethylchlorosilane−hexametyldisilazane−pyridine (2:1:2, v/v/v). This treatment was included to convert hydroxylated FA methyl esters into trimethylsilyl (Me_3Si) derivatives, which have better gas chromatographic behavior and mass spectroscopic properties as compared to the underivatized hydroxy esters.

GC-MS was performed with a Hewlett-Packard 5970B mass selective detector connected to a Hewlett-Packard model 5890 GC equipped with a Supelcowax capillary column (length, 30 m; film thickness, 0.25 μm). Helium at a flow rate of 30 cm/s was used as the carrier gas. Injections were made in the split mode using an inlet pressure of 27 psi and an initial column temperature of 150 °C. The temperature was raised at 3 °C/min until 250 °C. Authentic compounds were used for reference purposes, which included epoxy and hydroxy derivatives of 18:0, 18:1, and 18:2.

Statistical Analyses. Analysis of variance (ANOVA) was performed with the Statistica one-way ANOVA procedure (computer software "Statistica 6.0"). Least significant differences (LSD) at the 5% level of probability were used to separate treatment means. To show interactions between oil content, some FAs, and lipid classes values, Pearson correlation analysis was conducted.

RESULTS

Oil Content. The oil content among the accessions of wild oat species varied from 5.2% in *A. magna* to 9.4% in *A. canariensis* (**Table 1**). Species with high oil content (more than 8.5%) included *A. clauda*, *A. canariensis*, *A. murphyi*, *A. fatua,* *A. hirtula*, and *A. ludoviciana*. A large variance in oil content was displayed among accessions of species such as *A. canariensis* (8.6−9.4%), *A. barbata* (6.9−8.5%), *A. vaviloviana* (6.6−7.6%), *A. magna* (5.2−7.6%), *A. murphyi* (6.6−8.8%), and *A. fatua* (6.6−9.2%), while the three species *A. ludoviciana*, *A. hirtula*, and *A. sterilis* showed low variance in oil content among the analyzed accessions (8.4−8.7, 8.3−8.7, and

Table 2. Oil (Percent of Dry Weight Seed) and Lipid Content (Percent of Total Lipid) in Cultivated Oat[a]

cultivar	oil	PL	1,2-DAG	1,3-DAG	unknown lipid	FFA	TAG1	TAG2	TAG
Astor	5.2	16.1	1.1	2.1	1.3	2.0	1.3	1.1	74.9
Lodi	5.9	22.8	1.6	2.6	1.0	1.8	1.6	1.2	66.8
Borrus	6.4	16.3	1.0	1.9	1.0	2.0	1.3	1.1	75.1
Spear	4.6	13.9	0.9	1.9	1.0	2.7	1.3	1.1	77.0
Wright	6.3	11.9	0.9	1.9	0.8	0.5	1.0	0.9	81.8
Fakir	5.8	23.4	1.1	2.1	1.5	3.9	1.4	1.2	65.4
Allyur	4.1	12.6	1.4	2.9	1.7	4.1	1.5	0.9	74.6
Argamak	5.8	14.3	1.1	2.5	1.0	2.3	1.2	1.1	76.0
Torch	6.5	13.7	0.7	1.9	1.0	1.6	1.5	1.2	78.1
Kynon	8.3	15.6	1.2	2.8	1.8	3.3	1.7	1.2	72.5
mean	5.9	16.1	1.1	2.3	1.2	2.4	1.4	1.1	74.2
SEM	0.2	0.8	0.1	0.1	0.1	0.2	0.1	0.0	1.0
LSD	0.6	3.0	0.4	NS	0.6	1.3	NS	NS	5.6
F value	33.7***	16.2***	3.1*	1.7 NS	2.8*	6.3**	1.9 NS	0.9 NS	8.4***

[a] Eight covered and two naked (Torch and Kynon) cultivated oat accessions of *A. sativa* sp. (6*n*) were analyzed ($n = 3$). See **Table 1** for abbreviations. ***Significant at $p < 0.001$; **significant at $p < 0.01$; and *significant at $p < 0.05$. NS is nonsignificant.

Table 3. Oil (Percent of Dry Weight Seed) and Lipid Content (Percent of Total Lipid) in Wild (spp. *A. fatua*, *A. ludoviciana*, and *A. sterilis*, 6*n*) and Cultivated (*A. sativa*) Oat[a]

wild/cultivated	oil content	Pl	1,2-DAG	1,3-DAG	unknown lipid	FFA	TAG1	TAG2	TAG
wild	7.8 ± 0.2	15.4 ± 0.6	1.3 ± 0.1	1.9 ± 0.1	1.0 ± 0.1	1.5 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	77.1 ± 0.9
cultivated	5.9 ± 0.2	16.0 ± 0.7	1.1 ± 0.1	2.3 ± 0.1	1.4 ± 0.1	2.5 ± 0.2	1.4 ± 0.1	1.1 ± 0.0	74.2 ± 1.0
F value	47.5***	0.4 NS	1.3 NS	10.6**	4.2*	10.2**	13.2***	11.0**	4.3*

[a] See **Table 1** for abbreviations. ±standard error of mean. ***Significant at $p < 0.001$; **significant at $p < 0.01$; *significant at $p < 0.05$. NS is nonsignificant.

6.9–7.4%). The origin of accessions with high oil content (more than 8%) included a range of different regions such as Azerbaijan, Kazakhstan, Israel, Iran, Syria, Spain, Tunisia, and Egypt. Comparison of wild oat accessions of different ploidy levels and their oil content revealed that diploid species had significantly higher oil content (8.5%) than tetraploid and hexaploid oats (7.2 and 7.8%) (Supporting Information, Table 3).

The highest oil content among the cultivated oat analyzed, 8.3% (**Table 2**), was found in the naked cultivar Kynon (only two accessions of naked oat were analyzed). The remaining cultivars ranged in oil content from 4.1 to 6.5% with a mean of 4.9%. In comparison with wild hexaploid oats, cultivated hexaploid oats had significantly lower oil contents (**Table 3**).

Lipid Classes. The chromatography system used consistently resulted in separation of the oat groat oil into eight different lipid classes for each accession. The two major classes were triacylglycerols (TAG, R_f 0.76) and polar lipids (PL, R_f 0.01), where PL constituted a mix of phospholipids and glycolipids that cochromatographed. The highest content of PL among wild oat species was 23% (*A. magna* 4), and the lowest was 10.8% (*A. canariensis* 1), while the mean for all accessions was 15.9% (**Table 1**). Three accessions, *A. canariensis* 2, *A. magna* 4, and *A. sterilis* 1, contained more than 20% of PL (**Table 1**). It should be noted that a high percentage of PL was found both in accessions containing the highest (*A. canariensis* 2) and in those containing the lowest (*A. magna* 4) oil contents. Accessions within species differed considerably on this parameter with the widest variance found in *A. canariensis* (10.8–22.7%), *A. magna* (11.9–23%), and *A. sterilis* (11.8–21%). The TAG content ranged from 68 to 81.3% with a mean of 75.4%. A significant difference in TAG content between different accessions occurred in species such as *A. canariensis*, *A. vaviloviana*, *A. magna*, and *A. sterilis*. Tetraploid wild oat species had significantly more PL (16.8%) than diploid species (14.9%) and less TAG than hexaploid species (Supporting Information, Table 3).

The six minor lipid classes were identified as subclasses of diacylglycerols (DAGs) (1,2-DAG and 1,3-DAG, R_f 0.07 and 0.10), unknown lipid (R_f 0.13), free FAs (FFA, R_f 0.19), and subclasses of TAGs (TAG1 and 2, R_f 0.34 and 0.39). These minor classes made up around 8.6% of total lipids or 10.2% of neutral lipids, and the contribution of each individual lipid ranged from 0.5 to 4% of the content (**Table 1**). Diploid species contained more of 1,2-DAG and the unknown lipid (1.7 and 1.4%) than hexaploid species (1.3 and 1.0%) (Supporting Information, Table 3).

PL and TAG comprised on average 16.1 and 74.2% of lipids in cultivated oat, while minor lipid fractions made up 9.5% of total lipids and 11.4% of neutral lipids (**Table 2**). Cultivated oat also contained a significantly higher percentage of minor lipid classes as compared to wild species (**Table 3**).

FA Composition of Oil. Major FAs found in oat oil were 18:1 n-9, 18:2, and 16:0 with mean for wild oat of 40.5, 34.5, and 16.4% (**Table 4**) and for cultivated oats of 35.8, 40.4, and 15.9%, respectively (**Table 5**). In wild oat, 18:1 dominated over 18:2 with only three accessions, *A. magna* 1, *A. murphyi* 2, and *A. magna* 4, among 33 wild-growing accessions where 18:1 was less or equal to the content of 18:2. This is opposite to cultivated oat where only two accessions, Wright and Kynon, out of 10 accessions contained more 18:1 than 18:2 (**Table 5**). The highest content of 18:1 among wild oats was found in accession 3 of *A. hirtula* (43.8%) (**Table 4**), and the highest content of 18:2 among cultivars was found in Fakir and Allyur (43.1%) (**Table 5**). 18:3 was found in low amounts in all studied oats, 1.6% among wild accessions and not more than 2.1% among cultivars. In both wild and cultivated oats, there were significant differences between accessions on almost all FAs (**Tables 4** and **5**).

In addition to the common FAs found, several unusual FAs containing hydroxy or epoxy groups were identified in the oat

Table 4. FA Composition (Percentage of Total FAs) of Total Oil from Wild Oat Species of Different Ploidy Level (2*n*, 4*n*, and 6*n*)[a]

species	14:0	16:0	16:1	18:0	18:1 n-9	18:1 n-11	18:2	18:3	20:1	20:x[b]	7-OH-16:0	15-OH 18:2	epoxy 18:x[c]
A. clauda	0.0	16.5	0.2	1.2	39.2	1.1	35.9	1.2	0.9	0.3	0.1	3.2	0.3
A. longiglumis	0.2	17.1	0.2	1.8	41.2	0.4	33.6	1.2	1.0	0.3	0.1	3.5	0.4
A. canariensis 1	0.2	13.9	0.1	1.3	42.1	1.1	35.5	0.6	1.1	0.3	0.1	3.1	0.5
A. canariensis 2	0.2	16.3	0.2	1.6	40.4	0.9	34.2	1.1	1.2	0.4	0.1	3.1	0.5
A. hirtula 1	0.2	16.5	0.2	1.2	40.0	1.0	33.8	1.5	1.1	0.3	0.1	2.9	0.4
A. hirtula 2	0.3	16.6	0.2	1.5	38.9	0.9	35.0	1.3	1.2	0.2	0.1	2.8	0.3
A. hirtula 3	0.3	16.4	0.1	1.2	43.8	0.8	32.4	1.3	1.2	0.3	0.1	2.1	0.2
A. wiestii 1	0.3	16.7	0.2	1.4	41.1	0.9	33.7	1.2	1.0	0.3	0.1	2.7	0.5
A. wiestii 2	0.2	17.4	0.2	1.2	40.4	0.9	34.7	1.2	1.0	0.4	0.1	3.7	0.5
A. barbata 1	0.2	17.8	0.2	1.1	41.5	1.0	30.4	1.1	1.1	0.3	0.1	2.2	0.3
A. barbata 2	0.2	18.1	0.2	1.1	38.5	1.1	34.7	1.3	1.2	0.3	0.1	3.1	0.4
A. barbata 3	0.1	18.4	0.2	1.1	38.2	1.3	34.6	1.2	1.1	0.2	0.1	2.8	0.4
A. barbata 4	0.3	17.9	0.2	1.0	37.5	1.0	36.6	1.3	1.3	0.2	0.2	2.8	0.4
A. barbata 5	0.3	15.7	0.1	1.2	42.2	1.0	33.9	0.7	1.2	0.3	0.2	4.2	0.4
A. vaviloviana 1	0.4	17.3	0.2	1.0	36.6	1.0	35.1	1.6	1.1	0.2	0.1	4.1	0.4
A. vaviloviana 2	0.2	17.7	0.3	1.2	38.6	1.1	34.4	1.4	1.3	0.2	0.1	2.9	0.4
A. vaviloviana 3	0.2	17.7	0.1	1.2	41.1	1.0	33.0	1.3	1.1	0.3	0.1	3.2	0.5
A. magna 1	0.3	14.4	0.2	0.9	37.7	0.6	39.1	1.2	0.8	0.2	0.1	4.3	0.5
A. magna 2	0.2	15.1	0.1	1.0	41.7	0.9	36.0	1.0	0.9	0.3	0.1	2.2	0.4
A. magna 3	0.3	15.8	0.1	1.1	40.3	0.5	36.1	1.0	0.8	0.3	0.1	6.4	0.4
A. magna 4	0.2	14.9	0.2	0.9	38.2	1.0	38.1	1.3	1.1	0.2	0.1	3.0	0.4
A. murphyi 1	0.2	15.1	0.2	0.8	42.9	1.0	34.7	1.4	1.1	0.2	0.1	2.5	0.3
A. murphyi 2	0.2	14.7	0.2	1.2	37.3	1.1	37.4	1.2	1.0	0.3	0.0	2.8	0.3
A. fatua 1	0.2	14.0	0.2	1.3	40.4	0.9	36.4	1.4	0.9	0.2	0.1	2.6	0.4
A. fatua 2	0.3	15.2	0.2	1.3	40.4	0.9	36.2	1.4	1.1	0.2	0.1	2.5	0.4
A. fatua 3	0.2	15.9	0.2	1.4	42.7	0.9	33.9	1.3	1.0	0.5	0.0	3.5	0.3
A. ludoviciana 1	0.2	16.7	0.2	1.5	42.1	0.8	32.7	1.0	0.9	0.3	0.1	2.8	0.3
A. ludoviciana 2	0.1	17.1	0.2	1.4	41.1	1.0	33.9	1.1	1.0	0.6	0.1	3.9	0.3
A. ludoviciana 3	0.3	17.8	0.2	1.6	42.8	0.9	31.1	1.2	1.0	0.1	0.0	3.1	0.3
A. ludoviciana 4	0.2	17.1	0.2	1.3	39.5	0.9	34.4	1.3	1.0	0.4	0.1	3.7	0.6
A. sterilis 1	0.3	17.5	0.2	1.3	38.9	0.9	34.5	1.4	1.0	0.3	0.1	4.4	0.4
A. sterilis 2	0.2	18.1	0.2	1.2	41.9	1.0	32.2	1.1	1.0	0.2	0.1	2.7	0.4
A. sterilis 3	0.2	17.6	0.2	1.3	41.4	1.0	33.0	1.1	1.1	0.4	0.1	3.1	0.4
mean	0.2	16.4	0.2	1.2	40.5	0.9	34.5	1.2	1.1	0.3	0.3	3.2	0.4
SEM	0.0	0.2	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
LSD (0.05)	0.2	1.1	0.1	0.3	3.7	0.3	2.3	0.3	0.2	NS	0.1	NS	0.4
F value	2.0*	14.9***	2.4*	6.3***	2.4**	2.9***	5.8***	3.3***	3.8***	1.4 NS	2.0*	1.1 NS	2.5**

[a] ***Significant at $p < 0.001$; **significant at $p < 0.01$; and *significant at $p < 0.05$. NS is nonsignificant. [b] Includes FAs 20:0 and 20:2. [c] Includes FAs 9,10-epoxy-18:0, 9,10-epoxy-18:1, and 12,13-epoxy-18:1.

Table 5. FA Composition in Total Oil among Cultivars[a]

cultivar	14:0	16:0	16:1	18:0	18:1 n-9	18:1 n-11	18:2	18:3	20:1	20:x[b]	7-OH-16:0	15-OH 18:2	epoxy 18:x[c]
Astor	0.2	16.4	0.3	1.3	34.4	1.0	41.5	1.6	0.7	0.2	0.0	2.4	0.4
Lodi	0.2	16.4	0.3	1.4	34.5	1.0	40.9	1.6	0.7	0.2	0.1	2.3	0.5
Borrus	0.3	16.2	0.2	1.3	36.5	1.0	39.1	1.6	0.8	0.2	0.1	6.1	0.3
Spear	0.2	16.0	0.2	1.5	34.6	1.2	40.5	1.7	1.0	0.3	0.1	3.8	0.6
Wright	0.3	17.5	0.3	1.5	39.9	1.2	35.6	1.2	1.0	0.3	0.1	2.2	0.4
Fakir	0.2	15.1	0.1	1.3	35.1	1.1	43.1	1.5	0.5	0.1	0.1	3.0	0.4
Allyur	0.2	17.4	0.4	1.2	31.4	1.3	43.1	2.1	0.8	0.2	0.1	3.2	0.4
Argamak	0.2	16.0	0.1	1.6	36.2	1.0	40.8	1.3	0.8	0.3	0.1	3.1	0.3
Torch	0.2	16.1	0.3	1.7	36.7	0.9	39.0	1.3	0.7	0.3	0.1	1.9	0.3
Kynon	0.2	13.9	0.1	0.8	40.4	1.0	38.3	1.4	0.9	0.3	0.1	3.1	0.4
mean	0.2	15.9	0.2	1.4	35.8	1.1	40.4	1.5	0.8	0.2	0.1	3.1	0.4
SEM	0.0	0.2	0.0	0.1	0.5	0.0	0.4	0.1	0.0	0.0	0.0	0.3	0.0
LSD	NS	1.0	0.1	0.3	2.9	0.1	1.7	0.3	NS	NS	NS	3.7	NS
F value	1.6 NS	9.6***	6.6***	8.7***	9.2***	9.2***	16.1***	11.8***	1.9 NS	1.16 NS	0.7 NS	3.6*	1.0 NS

[a] ***Significant at $p < 0.001$; **significant at $p < 0.01$; and *significant at $p < 0.05$. NS is nonsignificant. [b] Includes FAs 20:0 and 20:2. [c] Includes FAs 9,10-epoxy-18:0, 9,10-epoxy-18:1, and 12,13-epoxy-18:1.

oil (**Tables 4** and **5**). Hydroxylated FAs included 15-hydroxy $18:2^{\Delta 9,12}$ (avenoleic acid) and 7-hydroxy 16:0. Because this is the first report of the presence of a 7-hydroxy 16:0 in cereals, a detailed analysis is presented below. Two additional hydroxylated FAs, 13-hydroxy $18:2^{\Delta 9,11}$ and 9-hydroxy $18:2^{\Delta 10,12}$, were found in the oat oil, but they were not analyzed for their content since this type of FAs did not survive the methylation using methanolic H_2SO_4. Epoxygenated FAs included 12,13-epoxy $18:1^{\Delta 9}$, 9,10-epoxy $18:1^{\Delta 12}$, and 9,10-epoxy 18:0. It should be noted that the methylation reagents involving H_2SO_4 caused ring opening of all epoxy FAs, which quantitatively were converted into hydroxy-metoxy derivatives. Therefore, the content of epoxy FA was given from these analyzed derivatives. With the exception of avenoleic acid, none of the unusual FAs made up more than 0.6% of total FAs. Avenoleic acid was found in amounts up to 6% with a mean value of about 3% in both wild

Table 6. FA Composition of Different Lipid Classes in Wild (6*n*) and Cultivated Oat[a]

lipid class		16:0	18:0	18:1 n-9	18:1n-11	18:2	18:3	20:1	7-OH-16:0	15-OH 18:2	epoxy 18:x[b]
PL	wild	20.3 ± 0.3	1.1 ± 0.0	20.6 ± 0.7	1.1 ± 0.0	40.9 ± 0.4	1.9 ± 0.0	0.4 ± 0.0	0.0 ± 0.0	12.7 ± 0.6	0.2 ± 0.0
	cult	19.3 ± 0.8	1.3 ± 0.1	18.6 ± 0.8	1.2 ± 0.1	46.0 ± 0.7	2.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	9.8 ± 0.8	0.4 ± 0.2
	F value	0.8	4.9*	2.1 NS	2.6 NS	35.7***	3.6*	1.6 NS	0.0	3.7*	0.9 NS
1,2-DAG	wild	15.7 ± 0.3	1.8 ± 0.1	37.9 ± 0.7	0.9 ± 0.0	33.8 ± 0.7	1.0 ± 0.1	0.7 ± 0.1	2.5 ± 0.4	0.0 ± 0.0	5.4 ± 0.4
	cult	13.7 ± 0.3	1.7 ± 0.1	30.6 ± 1.1	1.0 ± 0.1	36.3 ± 0.8	1.3 ± 0.1	0.6 ± 0.1	3.9 ± 0.5	0.0 ± 0.0	9.2 ± 1.5
	F value	21.8***	0.4 NS	12.7***	0.7 NS	31.5**	12.7*	1.1 NS	4.5*	0.0	5.4*
1,3-DAG	wild	20.6 ± 3.8	2.1 ± 0.7	33.6 ± 4.8	1.0 ± 0.3	32.2 ± 4.9	0.9 ± 0.3	1.1 ± 0.4	0.0 ± 0.0	1.2 ± 0.6	5.2 ± 5.4
	cult	17.6 ± 1.6	1.9 ± 0.5	31.1 ± 4.5	1.1 ± 0.2	37.4 ± 3.4	1.1 ± 0.4	0.9 ± 0.3	0.0 ± 0.0	1.0 ± 0.3	5.3 ± 4.6
	F value	18.1***	0.8 NS	6.8*	7.9**	29.5***	8.1**	6.5*	0.0	5.2*	0.0 NS
Unknown lipid	wild	17.1 ± 3.3	2.2 ± 0.8	28.8 ± 5.7	0.9 ± 0.4	34.2 ± 7.4	1.6 ± 1.0	0.9 ± 0.5	0.0 ± 0.0	2.0 ± 1.9	8.9 ± 5.0
	cult	14.8 ± 1.7	2.0 ± 0.6	26.2 ± 4.7	0.9 ± 0.3	36.1 ± 6.8	2.3 ± 1.0	0.9 ± 1.0	0.0 ± 0.0	2.0 ± 1.0	10.0 ± 7.1
	F value	13.1***	1.5 NS	5.0*	1.8 NS	1.6 NS	10.7**	0.0 NS	0.0	0.0 NS	0.8 NS
FFA	wild	28.2 ± 6.1	2.3 ± 0.8	27.6 ± 7.9	1.1 ± 0.6	32.1 ± 7.9	0.4 ± 0.4	1.1 ± 0.6	0.0 ± 0.0	0.1 ± 0.2	6.4 ± 6.8
	cult	25.7 ± 2.8	2.3 ± 0.9	20.4 ± 4.2	1.1 ± 0.4	43.5 ± 5.7	0.7 ± 0.4	0.7 ± 0.4	0.0 ± 0.0	0.1 ± 0.2	4.7 ± 3.0
	F value	4.6*	0.1 NS	22.5***	0.2 NS	53.4***	16.1***	12.7***	0.0	0.1 NS	1.7 NS
TAG1	wild	11.3 ± 2.0	1.5 ± 0.7	30.1 ± 4.3	0.6 ± 0.3	23.1 ± 5.6	0.6 ± 0.4	0.5 ± 0.4	0.0 ± 0.0	0.3 ± 0.4	31.2 ± 7.8
	cult	10.1 ± 1.2	1.2 ± 0.3	27.9 ± 4.7	0.8 ± 0.1	30.2 ± 3.8	0.9 ± 0.2	0.4 ± 0.2	0.0 ± 0.0	0.4 ± 0.3	27.4 ± 7.6
	F value	9.7**	3.9*	5.6*	5.7*	41.6***	15.8***	1.5 NS	0.0	2.4 NS	5.7*
TAG2	wild	17.4 ± 3.1	2.7 ± 0.9	28.7 ± 5.9	0.7 ± 0.4	17.2 ± 4.9	0.1 ± 0.3	1.1 ± 0.4	0.0 ± 0.0	0.3 ± 0.5	31.2 ± 11.4
	cult	15.3 ± 2.0	2.5 ± 1.3	28.3 ± 3.7	1.1 ± 0.2	22.4 ± 2.6	0.3 ± 0.2	1.0 ± 0.3	0.0 ± 0.0	0.4 ± 0.3	28.0 ± 6.9
	F value	11.8***	0.9 NS	0.1 NS	16.5***	30.4***	14.1***	4.1*	0.0	0.3 NS	2.2 NS
TAG	wild	16.0 ± 1.4	1.3 ± 0.3	45.4 ± 2.3	1.1 ± 0.2	32.1 ± 2.5	1.1 ± 0.3	1.2 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.9 ± 0.6
	cult	15.1 ± 1.6	1.4 ± 0.3	39.7 ± 2.6	1.2 ± 0.1	38.6 ± 2.3	1.4 ± 0.3	0.9 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.8 ± 0.4
	F value	9.1**	3.2 NS	133.8***	6.6*	158.8***	33.2***	57.5***	0.0	1.04 NS	0.7 NS

[a] ±Standard error of mean. ***Significant at $p < 0.001$; **significant at $p < 0.01$; *significant at $p < 0.05$. NS is nonsignificant. [b] Includes FAs 9,10-epoxy-18:0, 9,10-epoxy-18:1, and 12,13-epoxy-18:1.

and cultivated oat (**Tables 4** and **5**). Tetraploid wild oats had significantly lower amounts of 18:0 and 18:1 n-9 and higher amounts of 18:2 as compared to diploid and hexaploid wild oats (Supporting Information, Table 4).

FA Composition of Lipid Classes. The individual lipid classes identified on the TLC plate differed from each other by their specific FA compositions (**Tables 6** and Supporting Information, Table 5). In hexaploid wild oats (w) and cultivars (c), 16:0, 18:1, and 18:2 were the dominating FAs in all lipid classes except TAG1 and -2 (**Table 6**). The highest level of 16:0 was found in FFA$_w$ (28.2%), and the lowest level was found in TAG1$_c$ (10.1%); the highest level of 18:1 was found in TAG$_w$ (45.4%), and the lowest level was found in PL$_c$ (18.6%); and the highest level of 18:2 was found in Pl$_c$ (46.0%), and the lowest level was found in TAG2$_w$ (17.2%) (**Table 6**). PL$_w$ contained the highest amount of avenoleic acid (12.7%). As the only lipid class, 1,2-DAG contained the unique unusual FA 7-hydroxyhexadecanoic (2.5%$_w$ and 3.9%$_c$). 1,2-DAG also contained up to 9.2%$_c$ of epoxidized FA. Avenoleic acid was found in 1,3-DAG that also contained up to 5.3%$_c$ of epoxidized FA. The unknown lipid contained the highest amount of 18:3 (1.6%$_w$ and 2.3%$_c$) as well as 2%$_{w \text{ and } c}$ avenoleic acid and up to 10%$_c$ of epoxidized FAs. FFA contained up to 6.4%$_w$ of epoxidized FA. TAG1 had the lowest levels of 16:0 (11.3%$_w$, 10.1%$_c$), and TAG2$_w$ had the lowest 18:2 levels (17.2%). Both TAG1 and -2 had very high levels of epoxidized FAs (27.4−31.2%$_{w \text{ and } c}$). TAG$_{w \text{ and } c}$ had low levels of unusual FAs and high levels of 18:1.

Correlations. Correlations between the traits oil content, lipid classes, and main FAs of total oil are shown in **Table 7**. Oil content was positively correlated with 18:1 n-9 and negatively correlated with FFA, TAG1, 18:2, and 18:3. PL had a strong negative relationship with TAG and 18:1 n-9 and was positively associated with 18:2. With the exception for 1,2-DAG, most minor lipid classes showed positive correlation between each other. TAG was negatively correlated with 18:2 and 18:3 and all lipid classes except 1,2-DAG and positively correlated with 18:1 n-9. 18:1 n-9 was negatively correlated with both 18:2 and 18:3, while the two latter were highly positively associated.

Characterization of 7-Hydroxyhexadecanoic Acid. Analysis of the methyl ester-trimethylsilyl ether derivative of crystalline 7-hydroxyhexadecanoic acid by GC-MS showed a single peak having a *C* value of 18.03 and a mass spectrum showing a molecular ion at *m/z* 358 (0.5%) as well as prominent ions at *m/z* 343 ($M^+ - CH_3$; 7), 311 [M^+ − (CH_3 plus CH_3OH); 11], 231 [$Me_3SiO^+{=}CH{-}(CH_2)_5{-}COOCH_3$; 100], 229 [$Me_3SiO^+{=}CH{-}(CH_2)_8{-}CH_3$; 58], 202 [$M^+ - OHC{-}(CH_2)_8{-}CH_3$; 13], 127 (21), and 73 (Me_3SiO^+; 84). The FT-IR spectrum (film) of the methyl ester showed bands inter alia at 3318 (hydroxyl) and 1743 cm^{-1} (ester carbonyl). The ^1H NMR spectrum ($CDCl_3$, 400 MHz) of the methyl ester showed signals at δ 0.88 (3H, t, H-16), 1.27 (m, H-4, H-5, H-9 to 15), 1.44 (4H, m, H-6 and H-8), 1.64 (2H, m, H-3), 2.31 (2H, t, H-2), 3.58 (1H, m, H-7), and 3.67 (3H, s, methoxy).

The absolute configuration of 7-hydroxyhexadecanoic acid was determined by a stepwise degradation of the carbon chain followed by conversion of the resulting β-hydroxyester into a phenylpropionyl derivative using 2(*S*)-phenylpropionyl chloride. GC analysis of the reference 2(*S*)-phenylpropionyl derivative of methyl 3(*R*,*S*)-hydroxydodecanoate showed two peaks due to the 3(*R*)-2(*S*) and 3(*S*)-2(*S*) diastereomers eluting at 13.4 and 13.9 min, respectively, whereas the phenylpropionyl derivative obtained from 7-hydroxyhexadecanoic acid produced one peak cochromatographing with the 3(*R*)-2(*S*) derivative. Thus, the parent 7-hydroxyhexadecanoic acid had the "*R*" configuration at C-7.

Estimation of 7-Hydroxyhexadecanoic Acid in Different Cereal Seeds. Analyses of several cereals showed a relatively low content of 7-hydroxyhexadecanoic acid in the cultivars analyzed with levels of 23−31 μg/g recorded for barley seeds, 21−29 (oat), 20−29 (rye), and 14−26 μg/g (wheat).

Table 7. Correlation Matrix between the Content of Oil and Lipid Classes and the Content of Major FAs in the Total Oil from 6*n* Wild and Cultivated Oat[a]

	oil	PL	1,2-DAG	1,3-DAG	unknown lipid	FFA	TAG1	TAG2	TAG	16:0	18:0	18:1 n-9	18:2	18:3
oil	1.0000													
	p = —													
PL	−0.0459	1.0000												
	p = 0.730	*p* = —												
1,2-DAG	0.1413	0.0719	1.0000											
	p = 0.286	*p* = 0.588	*p* = —											
1,3-DAG	−0.1514	0.1698	**0.3725**	1.0000										
	p = 0.252	*p* = 0.199	***p* = 0.004**	*p* = —										
unknown lipid	−0.2177	0.1365	0.1476	**0.5087**	1.0000									
	p = 0.098	*p* = 0.303	*p* = 0.264	***p* = 0.000**	*p* = —									
FFA	**−0.3499**	0.2178	−0.0040	**0.4294**	**0.5255**	1.0000								
	***p* = 0.007**	*p* = 0.098	*p* = 0.976	***p* = 0.001**	***p* = 0.000**	*p* = —								
TAG1	**−0.3581**	0.1139	0.0484	**0.3773**	**0.4947**	**0.3809**	1.0000							
	***p* = 0.005**	*p* = 0.390	*p* = 0.716	***p* = 0.003**	***p* = 0.000**	***p* = 0.003**	*p* = —							
TAG2	−0.2556	0.1834	0.0026	0.2273	**0.4169**	**0.3201**	**0.5849**	1.0000						
	p = 0.051	*p* = 0.164	*p* = 0.984	*p* = 0.083	***p* = 0.001**	***p* = 0.013**	***p* = 0.000**	*p* = —						
TAG	0.1943	**−0.8907**	−0.2481	**−0.4750**	**−0.4372**	**−0.5309**	**−0.3926**	**−0.3849**	1.0000					
	p = 0.140	***p* = 0.000**	*p* = 0.058	***p* = 0.000**	***p* = 0.000**	***p* = 0.000**	***p* = 0.002**	***p* = 0.003**	*p* = —					
16:0	−0.0923	0.0095	−0.0361	−0.1124	**−0.2618**	−0.0529	**−0.3275**	**−0.2856**	0.0921	1.0000				
	p = 0.487	*p* = 0.943	*p* = 0.786	*p* = 0.397	***p* = 0.045**	*p* = 0.691	***p* = 0.011**	***p* = 0.028**	*p* = 0.488	*p* = —				
18:0	−0.0406	−0.0333	−0.0880	**−0.2560**	**−0.4244**	−0.1350	−0.1024	0.0864	0.1264	0.0921	1.0000			
	p = 0.760	*p* = 0.802	*p* = 0.507	***p* = 0.050**	***p* = 0.000**	*p* = 0.308	*p* = 0.440	*p* = 0.515	*p* = 0.340	*p* = 0.488	*p* = —			
18:1 n-9	**0.7248**	**−0.2783**	0.0363	**−0.3440**	**−0.3276**	**−0.4525**	**−0.3387**	**−0.3130**	**0.4430**	0.0663	0.0669	1.0000		
	***p* = 0.000**	***p* = 0.033**	*p* = 0.785	***p* = 0.008**	***p* = 0.011**	***p* = 0.000**	***p* = 0.009**	***p* = 0.016**	***p* = 0.000**	*p* = 0.618	*p* = 0.615	*p* = —		
18:2	**−0.7277**	**0.3030**	−0.0293	**0.3867**	**0.4156**	**0.5017**	**0.4627**	**0.4428**	**−0.5045**	**−0.2873**	−0.1154	**−0.9168**	1.0000	
	***p* = 0.000**	***p* = 0.020**	*p* = 0.826	***p* = 0.002**	***p* = 0.000**	***p* = 0.000**	***p* = 0.000**	***p* = 0.000**	***p* = 0.000**	***p* = 0.027**	*p* = 0.384	***p* = 0.000**	*p* = —	
18:3	**−0.6680**	0.1081	−0.0068	**0.2884**	**0.3478**	**0.4747**	**0.3095**	0.2096	**−0.3023**	−0.0589	−0.2416	**−0.8099**	**0.7622**	1.0000
	***p* = 0.000**	*p* = 0.415	*p* = 0.959	***p* = 0.027**	***p* = 0.007**	***p* = 0.000**	***p* = 0.017**	*p* = 0.111	***p* = 0.020**	*p* = 0.658	*p* = 0.065	***p* = 0.000**	***p* = 0.000**	*p* = —

[a] Values were calculated from merged data for both wild and cultivated oats. Numbers in bold are significant. See **Table 1** for abbreviations.

DISCUSSION

Oil Content. Our data on oil content showed high variation among wild oat accessions of different ploidity as well as in hexaploid cultivars. Oil content is to a great extent influenced by the genotype of oat, and a wide genetic diversity of this trait has also been shown to exist among wild species of oats as well as among cultivars. Frey and Hammond (*18*) analyzed diploid, tetraploid, and hexaploid wild oats and reported ranges in oil content of 3.5−9.0, 5.5−8.0, and 2.0−11.0%, respectively, while analyses of Canadian oat cultivars showed a range in oil content from 4.2 to 11.8% (*19*). Welsh and Legget (*10*) analyzed a greenhouse-cultivated oat material consisting of six cultivars and 10 wild oat species of which six, *A. sterilis*, *A. longiglumis*, *A. canariensis*, *A. hirtula*, *A. barbata*, and *A. murphyi*, were the same as in this study. *A. sterilis* and *A. barbata* had higher oil contents than in the present study, while the remaining four species had similar values. However, in two other species analyzed, *A. ventricosa* and *A. eriantha*, the oil content was very high, 12.2 and 13.7%, respectively, and the range between all wild species was higher than in the present study (6.9−13.7%) (*10*). Because the alleles for high oil content from *A. sativa* and *A. sterilis* are complementary, a program to raise the oil content by using recurrent selection was carried out and resulted in increases in groat oil concentration of up to 18% in a relatively short time (*5*). It was concluded that the potential of further increasing the groat oil content through continued recurrent selection is not exhausted but that it is important to keep a focus on possible changes in grain qualities as well as agronomic performances in the population (*5, 20*). The present study has shown that several other diploid and hexaploid wild species may as well hold interesting germplasm with the potential of raising the oil content.

FA Composition. The FA composition in wild species and cultivars of oat was in line with what has been reported (*10, 20*). In all but three accessions of the wild species, the levels of 18:1 were higher than 18:2. Interestingly, these three accessions were all tetraploid, which had significantly less oil content as compared to diploid and hexaploid oats. Previous observations have also shown that more efficient oil accumulation is accompanied with an increased amount of 18:0 and 18:1 and decreased amounts of 16:0, 18:2, and 18:3 (*11, 20*). In contrast to wild species, a majority of the cultivated oats had higher 18:2 than 18:1 FAs as well as much lower oil contents as compared with the wild species. In addition, the higher 18:2 levels were accompanied by marginally increased 18:3 levels. However, it should be noted that levels of 18:3 were very low in all wild species as well as cultivars investigated in this study. Indeed, most published reports on FA composition in oat show similar low levels of 18:3 as in this study (*10, 11, 20−22*). Holland et al. (*20*) also observed that while significant genetic variation exists for each FA in oat, this does not apply for 18:3. This suggests a lack of potential for substantially increasing 18:3 levels through breeding work using the existing oat gene pool.

Several unusual FAs identified by mass spectrometry as hydroxy and epoxy derivatives of 16:0, 18:0, 18:1, and 18:2 were present in the oat oil. The recently identified hydroxy FA avenoleic acid seems to be unique for oat among cereals and has been shown to be attached to an unusual galactolipid (*16, 23*). In this study, avenoleic acid was mainly found among PL since the solvent system used included galactolipids in this lipid class. Furthermore, a novel hydroxy FA, 7-hydroxyhexadecanoic acid, was identified for the first time in cereals. Its confirmed "*R*" configuration supports that its presence is a result of enzymatic activity. This FA had previously only been reported in a study of archeological finds from native Americans and traced to the paint made from the insect *Tuchardiella lurrae* (*24*). In contrast to

avenoleic acid, the novel 7-hydroxyhexadecanoic acid was not only present in low levels in the total oil of oat but also found at low levels in barley, rye, and wheat as well.

Lipid Classes. TAG made up a greater part of the oil in the oat grain. However, while in typical oil crops such as rapeseed and sunflower the seed oil is made up of more than 95% of TAGs and only a few percentages of PLs, in the groat oil of the cereal oat, PLs can constitute more than 20% of the oils (this study and refs *11*, *25*, and *26*). The high percentage of PLs in the oat groat oil thus distinguishes it from the oil composition in most oil seed crops.

TAGs were also highly negatively correlated to PLs and to most of the other analyzed lipid classes in wild and cultivated hexaploid oat, while there was only a weak positive connection between TAGs and oil content. However, in the tetraploid oats, which had lower oil content as compared with the hexaploid and diploid ones, we found significantly less TAG than in hexaploid and significantly more PL than in diploid oat (Supporting Information, Table 3). Indirect support of positive association between oil content and TAG could also be found in the positive correlation between TAG and 18:1 and between oil content and 18:1 as well as that a significantly higher percentage of TAG was found in wild oats that overall showed a higher oil content than in the cultivars. In a recent report on lipid deposition during oat groat development, it was shown that a higher oil content in the "high-oil" variety Matilda (10%) was due to higher oil content in the endosperm (*26*). In addition, the endosperm deposited oil was to a much larger extent in the form of TAG in the "high-oil" variety than in the "low-oil" variety. The above results suggest that not only the trait for high oil content but also the ability of accumulating the oil as mainly TAG and perhaps also to deposit the oil in the endosperm are highly influenced by the genotype. Regarding major FAs, 18:1 and 18:2 FAs were at similar levels in TAG in cultivated oat, while the former comprised almost half of all FAs in TAG in wild oats. The major FA in PL was 18:2. These results are also in accordance with what has been previously reported (*19*).

Beside the two dominating lipid classes, a number of minor classes in the oat oil were easily separated and detected on the TLC plate. On the basis of comparison with reference samples, they were identified as two species of DAG (1,2- and 1,3-DAG), an unknown lipid, FFA, and two additional TAG species (TAG1 and TAG2). Additional and more detailed analyses will be needed to further unravel the molecular structure of the unknown lipid, TAG1, and TAG2 species, respectively.

Interestingly, the novel 7-hydroxyhexadecanoic acid was exclusively found in 1,2-DAG where it contributed to up to 4% of the FA composition. The unknown lipid separated in the TLC system close to 1,3-DAG but was distinguished from this lipid by containing more avenoleic acid and 18:3 FA and less 16:0. The two minor TAG classes (TAG1 and TAG2) differentiated from other classes in that they contained more than 30% of epoxidized 18:0 and 18:1 FAs, which made them migrate differently than TAG containing common FAs. These classes had also comparably much lower amounts of 18:2, which suggests that this FA was the target molecule for enzymes such as peroxygenase, lipoxygenase, and lipoperoxidase (*27*).

It can be concluded from the present study that wild oat species of different ploidy levels carry a great variation in not only the oil content but also in the parameters such as FA and lipid composition, which are important traits that influence the oat oil quality. Previous work has shown the great potential in breeding for cultivars with different oil contents. The present study and others now clearly indicate the potential for developing cultivars having a range of different oil qualities. Such cultivars should have a potential in further diversifying the use of oat as a food source.

Supporting Information Available: Tables of information on the accessions of wild oat species and oat cultivars included in this study (Tables 1 and 2). Tables of the percentage of oil and lipid classes and the FA composition in total oil of each individual ploidy level (2*n*, 4*n*, and 6*n*) of wild oats (Tables 3 and 4). Table of the FA composition of the lipid classes as a mean of the three ploidy levels (Table 5). This material is available free of charge via the Internet at http://pubs.acs.org.

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