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# Genetics, Characteristics, and Utilization of Oil in Caryopses of Oat Species[1]

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### ABSTRACT

For oats to be an economically feasible oilseed crop in Iowa, the oil percentage would have to be increased to ca. 16%. A survey of the oil percentage in 445 oat cultivars and collections gave a range of 2.0-11.0%. The oil percentage was only slightly affected by growing oats in 5 different locations in Iowa. Inheritance studies indicated that oil percentage was inherited polygenically, and there was a tendency for high oil percentage to be partially dominant. Analysis of 64 cultivars and collections showed a wide variation of fatty acid composition: palmitic, 14-23%; stearic, < 1-4%; oleic, 29-53%; linoleic, 24-48%; linolenic, < 1-5%. The oil percentage was positively correlated with oleic acid and negatively correlated with linoleic and linolenic acids. Oats contained a lipase that made extraction of oil with low acid values difficult. The lipase was strongly affected by moisture and was most active in oat doughs containing 25-50% moisture. There was at least a 20-fold variation in lipase activity in oat cultivars and collections. Whole oats may be kept in dry storage for several years without significant lipolysis, but in broken or crushed caryopses, lipolysis occurs even at low moisture levels. The lipase may be inactivated by heat or 95% ethanol treatments.


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### INDEX



### INTRODUCTION

A high proportion of the arable land in midwestern United States is devoted to the cultivation of corn and soybeans. This lack of crop diversity makes midwestern agriculture especially vulnerable to epidemics of insects and diseases, but it persists because no other crop adapted to the midwestern USA is as profitable as corn and soybeans. During the first half of the twentieth century, oats were a major crop in Iowa, but recently, they have proved less profitable than corn and soybeans. Currently, oats are grown with poor field husbandry on land that is too steep to be sown in corn or soybeans. If oats could be made more profitable, their acreage would be expanded, and Iowa agriculture would enjoy greater diversity. One way to make oats more profitable might be to grow them as an oilseed crop.

Traditionally, oats have not been used as a source of edible oil because: a) the amount of oil in the caryopses of commercial oat cultivars is quite low, generally ranging from 3.9-8.5%, according to Brown, et al., (1); and b) a potent lipase enzyme is extracted with the oat oil which causes rapid hydrolysis and deterioration of the oil quality, according to Hutchinson and Martin (2). However, Brown and Craddock (3) have analyzed lines from the World Oat Collection and found 25 lines with < 4.0% oil, 63 lines with > 10.0% oil, and 5 with > 11.0% oil. These results suggest that there is considerable genetic variation for this trait among cultivated oat lines. Baker and McKenzie (1), using crosses between low, medium, and high oil varieties, found that the heritability of oil percentage in oat caryopses varied from 68-93% except in 1 cross. These values predict that selection for oil percentage in oats would be accomplished with ease.

We undertook this study to obtain information about oat oil and its biology for use in deciding whether it would be possible to breed oats with sufficiently high oil content to make extraction profitable, and to study some problems associated with extracting the oil.

## MATERIALS AND METHODS

### Oat Materials

The materials used for our studies were caryopses samples from oat genotypes representing several oat species (*Avena* spp.). For the survey of oil percentages, we used samples from 5 diploid species, *A. brevis, A. ludoviciana, A. pilosa, A. strigosa,* and *A. wiestei,* 1 tetraploid species, *A. barbata,* and 3 hexaploid species, *A. fatua, A. sativa,* and *A. sterilis.* These 445 collections and cultivars were grown in unreplicated hill plots spaced 75 cm apart in perpendicular directions with 80 plots per row. Three check cultivars of *A. sativa* were sown systematically at 20-plot intervals. Each plot was harvested and threshed when mature. From each plot, we dehulled 20 seeds, and this sample of caryopses was used to determine the oil content via the nuclear magnetic resonance (NMR) method of Conway and Earle (5).

To determine the stability of oat genotypes in oil percentage, we used 6 cultivars, 'Multiline E73,' 'Grundy,' 'Clintford,' 'Otee,' 'O'Brien,' and 'Dal,' each grown at 6 locations in Iowa. Samples (5 g) of dehulled seeds of these cultivars were assayed for oil on a replicate basis. Five experiments had 3 replicates, and the other had only 2. A pooled analysis of variance was conducted on the data collected in this experiment.

For the inheritance study on oil percentage, we used 60-80 $F_2$ derived lines from each of 3 interspecific oat crosses and 10-20 lines from each parent. Crosses used were 'CI 8044' x 'B 439', (Iowa accession numbers), 'Clintford' x 'B 439,' and 'Clintford' x 'B 440.' 'CI 8044' and 'Clintford' are *A. sativa* cultivars, and 'B 439' and 'B 440' are *A. sterilis* accessions. The $F_2$ derived lines were in $F_3$ when analyzed, and the intracultivar lines were derived as single plant progenies. $F_2$ derived and parental lines were in hill plots spaced 30 cm apart in perpendicular directions in a randomized design with 1 replicate. The block of plots was surrounded by 2 rows of hills to provide competition for all test plots. Twenty caryopsis samples of these lines were used for oil determinations.

The oat plants used in all of these studies were grown on highly fertile soil, and they were sprayed at weekly intervals from anthesis to maturity with a fungicide to control foliar diseases.

### Laboratory Methods

The fatty acid composition of oats cultivars and collections was determined on 10 caryopses of a cultivar after they were dried in a vacuum oven at 105 C and crushed. The crushed caryopses were extracted with 1 ml hexane, and an aliquot of the hexane solution was treated with 1 N sodium methoxide solution in methanol to convert the oil to methyl esters. The methyl esters were analyzed by gas chromatography on a 2 m EGSS-X column at 180 C.

To screen oat cultivars and collections for lipase activity, 3 caryopses from each type were pressed into tributyrin agar prepared according to Ellinghausen and Sandvik (6). After 24 hr at room temperature (ca. 25 C), the size of the clear zones around the caryopses was judged visually on a scale from 0-4. Zero represented no visible clear zone, and 4 represented a clear zone ca. 2 mm wide.

For a more exact determination of the lipase activity of oats, 5 caryopses were weighed and crushed with a glass rod. Enough water was added to give 30% by wt of the caryopses, and 50 $\mu$l of a 10% solution of soybean oil in heptane was added. After mixing thoroughly, the oat paste was left at 37 C for 1 hr; then 10 ml chloroform:methanol (2:1, v:v) was added and mixed thoroughly to stop the reaction and extract the lipid. The chloroform:methanol extract was centrifuged, and the supernatant was collected, treated with 2.0 ml water, cooled in ice water, and centrifuged again. An aliquot of the chloroform layer was made to 4 ml with chloroform and heptane so that the ratio of heptane to chloroform in the final mixture was 1:1 (v:v). The free fatty acid content in the mixture was determined by the cobalt soap method of Novak (7).

In one study, we tested the relationship of grain moisture content and lipase activity. To obtain several grain moisture levels for storage, caryopses were placed in desiccators containing anhydrous $CaSO_4$ and saturated solutions of $NaNO_3$, $CaCl_2$ and $MgCl_2$. The moisture level obtained after 4 months was determined by drying a sample at 110 C overnight in a vacuum oven.

To test the effect of lipase inactivation procedures on the development of free fatty acids during fat extraction, 50 g of caryopses were ground in a Wiley mill and extracted immediately in a Soxhlet apparatus with heptane. The solvent was evaporated, and the acid value of the residue was determined by AOCS method Cd 3a-63 (8).

TABLE I

Economic Return to Iowa Farmers Growing Oats, Corn, and Soybeans under Typical Conditions

| Crop | Yield (lb/acre) | Price ($/lb) | Production cost ($/lb) | Net profit ($/acre) |
|---|---|---|---|---|
| Oats | 2560 | .039 | .038 | 2.50 |
| Corn | 6160 | .036 | .029 | 43.12 |
| Soybeans | 2100 | .092 | .057 | 73.50 |

## RESULTS AND DISCUSSION

### The Economics of Producing Oat Oil

The exact percentage of oil required to make oats profitable as an oilseed crop is an elusive figure, because the costs of production vary. Table I shows the return per acre for Iowa farmers from growing corn, oats, and soybeans under typical conditions and when the only income was from selling grain at standard market prices (R.N. Wisner, personal communication, 1974). Of course, corn and soybeans are preferred because they produce more net income per acre than do oats. One way to make oat grain command a higher price would be to increase the oil and protein content of the seed without corresponding increases in production costs or loss in grain yield. The protein content of oats has been increased from a typical value of 17% to 21% in some new cultivars, without a sacrifice in grain yield. Oat protein has the best biological value of the cereal proteins (9), but it is not as good as soy protein. On the other hand, oat protein does not have the toxic factors that soy protein has. The composition of oat oil suggests that it should be more stable than soybean oil. If one assumes that oat protein is worth about as much as soy protein, and that oat oil is worth as much as corn oil, the value of an oat cultivar with 21% protein and 17% oil would make oats as profitable as soybeans under typical conditions in Iowa. Most commercial varieties of oats now contain about 4-6% oil.

### Survey of Oil Content of Oat Species

The results of our survey of oil percentages in oat cultivars and collections are shown in Table II. Among the diploid species, the range of oil percentages was 3.5-9.0%, among tetraploid species, it was 5.5-8.0%, and among hexaploid species, it was 2.0-11.0%. The major portion of the hexaploid samples belonged to the species *A. sterilis*, a weedy species of oat that grows in the waste areas sur-

TABLE II

Ranges of Caryopsis Oil Percentages of Diploid, Tetraploid, and Hexaploid Oat Cultivars and Collections

| Ploidy level | Number examined | Oil range (%) |
|---|---|---|
| Diploid | 48 | 3.5- 9.0 |
| Tetraploid | 6 | 5.5- 8.0 |
| Hexaploid | 391[a] | 2.0-11.0 |

[a]Of these 330 were *Avena sterilis*.

TABLE III

Analysis of Variance of Oil Percentage in Caryopses of Oat Cultivars Grown at Different Sites in Iowa

| Source | Degrees of freedom | Mean squares |
|---|---|---|
| Location | 5 | 0.914[a] |
| Cultivar | 5 | 11.972[a] |
| Replicate/location | 11 | 0.050 |
| Location/cultivar | 25 | 0.095[a] |
| Error | 55 | 0.037 |

[a]Significant at ($P<0.01$).

rounding the Mediterranean Sea, and especially in Israel. The range of caryopsis oil percentages for this species was 4.5-11.0%, with modal percentages of 7.0 and 8.5%. This species can be crossed freely with *A. sativa*, the common cultivated oat, so the availability of this vast new pool of germplasm may make it possible to increase the maximum oil percentage in oat caryopses considerably.

## Effect of Environment on Oil Percentage

Judging from the analysis of variance given in Table III, oil percentage in oat caryopses is largely determined by the genetic effect, but it is modified slightly by environmental effects.

## Inheritance of Oil Content

Frequency distributions of the oil percentages for the $F_2$ derived and parental lines are shown in Table IV. The frequency distribution indicates that the oil percentage is inherited polygenically, and that there is a tendency for high oil percentage to be partially dominant. Transgressive segregation for oil percentage occurred in 1 cross ('CI 8044' x 'B 439'), with 3 lines having higher oil percentages than the highest parental line. There was no significant correlation between oil percentage and groat wt, heading date, or plant ht.

## Variation in Fatty Acid Composition of Oat Oil

Sixty-four oat cultivars and collections were analyzed for fatty acid composition. The major fatty acids of oat oil were palmitic, stearic, oleic, linoleic, and linolenic acids. Minor amounts (< 0.1%) of lauric, myristic, palmitoleic, and arachidic acids also were detected in many samples. Figure 1 shows a frequency distribution for the amounts of the major fatty acids in these oat samples. There was considerable variation for all fatty acids except stearic. Most of the samples have less than 2.0% linolenic acid. The low level of this triunsaturated acid should contribute to the flavor stability of oat oil. Probably, oat oil with a wide range of compositions could be produced. Correlation coefficients calculated from these data showed palmitic acid to be negatively correlated with linoleic acid (-0.52), and linolenic acid positively correlated with linoleic acid (+0.79).

Table V shows the correlations of oil percentage with the various fatty acids using samples within *A. sterilis*. Oleic acid was positively correlated with high oil percentage while linoleic and linolenic are negatively correlated. All of these correlations were significant ($P < 0.01$). Thus, in selecting for high oil, an oil with high oleic and low linoleic and linolenic acids would be favored.

## Oat Lipase

The tributyrin test used to screen 352 oat collections and cultivars for lipase activity showed 58 samples with no lipase activity after 24 hr, 217 samples with a detectable zone of clearing, 65 samples with an obvious clear zone, and 12 samples with a large clear zone. However, there was little association between these results and those from the quantitative test for lipase activity based on the cobalt soap method. The colorimetric method revealed about a 20-fold variation in lipase activity, from 10-210 μM/g/hr of fatty acid released. We have no strain of oats with zero lipase activity, but considerable variation in lipase level does seem to exist; thus, reduction of oat lipase by breeding might be possible.

The lipase activity of oats is affected strongly by the moisture level of the grain. Martin and Peers (10) reported that oat lipase was located on the surface of the caryopses and might be removed by scrubbing. The lipase they obtained from the surface of the caryopses was active against soluble esters such as tributyrin and triacetin, but would not work against long chain triglycerides except in oat dough. Berner and Hammond (11) found that this lipase was active against long chain triglycerides emulsified with gum acacia. In this study, we found that lipase activity in oat dough was strongly influenced by the amount of water included in the dough, even if the triglycerides were added as a gum acacia emulsion. Additions of water equal to

TABLE IV

Frequency Distribution and Means of Oil Percentages for *A. sativa* Cultivars 'C.I. 8044' and 'Clintford,' *A. sterilis* Accession 'B439' and 'B440,' and Segregates from 3 Interspecific Crosses

| Class center (%) | 'C.I. 8044' X 'B439' | | | 'Clintford' X 'B439' | | | 'Clintford' X 'B440' | | |
|---|---|---|---|---|---|---|---|---|---|
| | $P_1$ | $P_2$ | Cross | $P_1$ | $P_2$ | Cross | $P_1$ | $P_2$ | Cross |
| 4.75 | 1 | | | | | | | | |
| 5.25 | 2 | | 3 | 9 | | 1 | 9 | | |
| 5.75 | 17 | | 5 | 10 | | 1 | 10 | | |
| 6.25 | | 1 | 22 | 1 | 2 | 7 | 1 | | 6 |
| 6.75 | | 1 | 22 | | 2 | 19 | | | 12 |
| 7.25 | | 3 | 19 | | 6 | 13 | | | 17 |
| 7.75 | | 5 | 5 | | 4 | 11 | | 1 | 18 |
| 8.25 | | | 3 | | 5 | 5 | | 7 | 20 |
| 8.75 | | | | | 1 | 1 | | 8 | 4 |
| 9.25 | | | | | | | | 1 | 1 |
| 9.75 | | | | | | | | 2 | 1 |
| Mean | 5.65 | 7.35 | 6.73 | 5.55 | 7.53 | 7.11 | 5.55 | 8.64 | 7.60 |

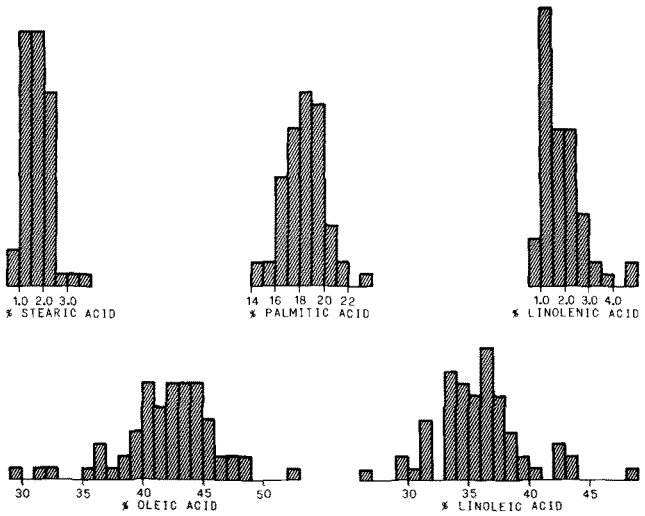


FIG. 1. Frequency distribution of the percentage of the major fatty acids in 64 oat cultivars and collections. The shortest bar in each distribution represents 1 cultivar or collection.

TABLE V

Correlation of Oil Content with Fatty Acids of Oat Oil

| Oil percent with | Correlation | Mean (%)[a] | Standard deviation of mean |
|---|---|---|---|
| Palmitic acid | -0.08 | 18.9 | 0.23 |
| Stearic acid | 0.03 | 1.7 | 0.07 |
| Oleic acid | 0.37[b] | 42.2 | 0.61 |
| Linoleic acid | -0.38[b] | 35.6 | 0.58 |
| Linolenic acid | -0.36[b] | 1.8 | 0.13 |

[a]Oil percentage mean = 8.6.

[b]Significantly different ($P < 0.01$) from zero; d.f. = 44.

25-50% of the wt of the caryopses increased lipase activity, but at 75-100%, the activity was inhibited. Lipase could not be detected at water levels above 100% of the wt of the caryopses. It may be that the effect of moisture on the lipase explains the lack of correlation between the tributyrin and colorimetric lipase tests. The latter should give a clearer indication of the lipase activity that would be encountered during the extraction of oat oil.

A typical value for the free fatty acid in whole caryopses stored in our laboratory was 15 μM/g, if the extraction was made with chloroform:methanol, but 6 μM/g if extracted with heptane. This indicates that some of the fatty acid is bound to the nonlipid portion of the caryopses. If the caryopses contain ca. 7% oil, this would amount to ca. 6 and 2.5% free fatty acid as oleic acid. It is not clear whether this value would remain the same or drop proportionately if the oil content were raised.

Figure 2 shows how the free fatty acid content of oat caryopses changed when they were held at different humidities for 4 months. The 7.5% moisture was obtained by storage in the atmosphere of our laboratory in the winter. Values higher than this are likely to lead to higher free fatty acids for whole caryopses, and even 7.5% moisture is not good for broken caryopses. Breaking the caryopses probably allows better contact of enzyme and substrate. Grinding caryopses will allow an even faster increase in free fatty acid. Caryopses stored in contact with the laboratory atmosphere doubled their free fatty acid overnight after grinding. Oats generally are stored at less than 12% moisture to prevent mold growth; however, there was no obvious mold growth on the samples used in Figure 2, except at the highest moisture level and at the end of the storage period. Oats with intact hulls may be stored for several years at the humidities encountered in dry storage in Iowa without significant increase in free fatty acid level.

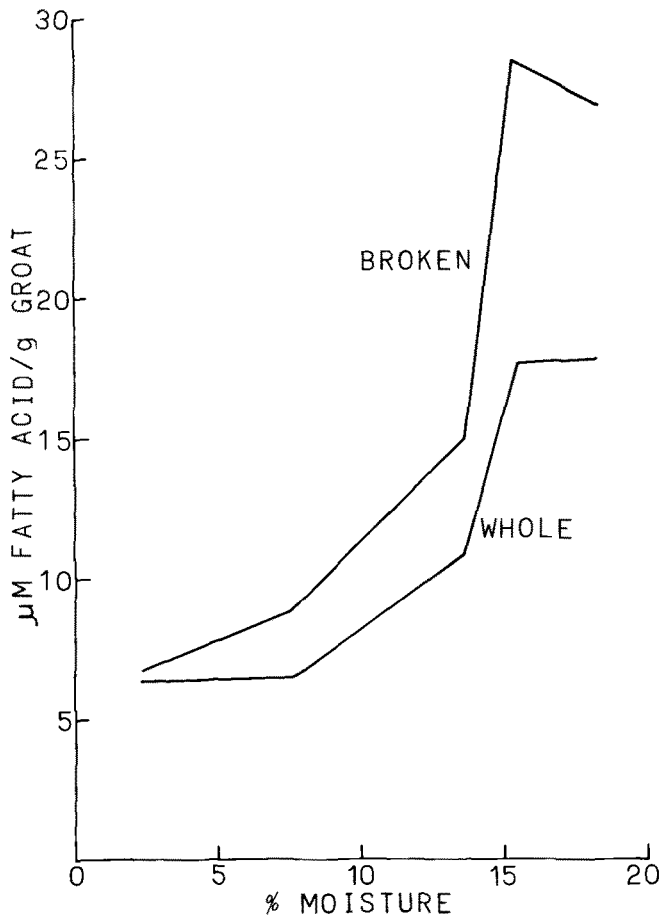


FIG. 2. The free fatty acid content of oat caryopses (groats) stored at various moisture levels for 4 months.

TABLE VI

Percentage of Free Fatty Acid as Oleic Acid in Oil Extracted from Oat Caryopses Given Various Treatments to Destroy Lipase

| | % |
|---|---|
| Control | 7.7 |
| Steamed whole caryopses (30 min) | 4.6 |
| Tumbled wet in ball mill (80 min) and washed | 6.9 |
| Tumbled wet in ball mill (60 min) with sand | 7.9 |
| Ground with ethanol; extracted with hexane | 4.0 |
| Ground with acetone; extracted with hexane | 6.1 |
| Soaked in 1 N HCl (30 min); washed and dried | 6.7 |
| Boiled (5 min); dried in vacuum oven | 2.6 |

Various ways of inactivating lipase could be used before grinding and extraction. Results from such techniques are shown in Table VI. These extractions were made by using a Soxhlet apparatus. Perhaps very rapid extraction would give sufficiently low free fatty acid content, even without lipase inactivation. The most effective treatment was boiling the caryopses in water, followed by vacuum drying. Steaming whole caryopses was less effective than boiling. In producing rolled oats lipase is destroyed by steam treatment during rolling, a procedure that might be the best for extraction. However, we could not simulate this procedure in our laboratory. The second most effective treatment was grinding in 95% ethanol, followed by extraction with heptane. Heptane was used because 95% ethanol is a poor solvent for oil. Possibly, this extraction method also extracted acid materials other than true fatty acid. Acetone seemed less effective than ethanol in inactivating the lipase. Treatment with acid to destroy lipase, a procedure reported by Hutchinson, et al., (12) was ineffective. Tumbling caryopses with or without sand to scrub off the surface lipase also was ineffective.

These results indicate that, even if lipase cannot be removed by breeding, it should be possible to extract oat oil with low free fatty acids if the oats are stored properly and if the caryopses are treated with heat or 95% ethanol during extraction.

## ACKNOWLEDGMENTS

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