Lipids in grain tissues of oat (Avena sativa): differences in content, time of deposition, and fatty acid composition


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Abstract

Oat (Avena sativa) is unusual in comparison with other cereals since there are varieties with up to 18% oil content. The lipid content and fatty acid composition in different parts of the grain during seed development were characterized in cultivars Freja (6% oil) and Matilda (10% oil), using thin-layer and gas chromatography, and light and electron microscopy. The majority of lipids (86–90%) were found in the endosperm. Ninety-five per cent of the higher oil content of cv. Matilda compared with cv. Freja was due to increased oil content of the endosperm. Up to 84% of the lipids were deposited during the first half of seed development, when seeds were still green with a milky endosperm. Microscopy studies revealed that whereas oil bodies of the embryo and scutellum still contained a discrete shape upon grain maturation, oil bodies of the endosperms fused upon maturation and formed smears of oil.

Key words: Avena sativa, embryo, endosperm, grain development, oat lipids, oil body.

Introduction

Cereal grains are generally low in oil content, and the oil is mostly confined to the embryo and scutellum. In kernels of high-oil maize (Zea mays), the oil-rich embryo and scutellum are enlarged, leading to a higher oil content than in traditional varieties (Alexander and Seif, 1963). Oat (Avena sativa) grains are relatively rich in oil compared with other cereals and can vary from 3% to 11% of grain weight in different cultivars, with lines containing up to 18% (Frey and Holland, 1999). Unlike other cereals, the major portion of the oil in oat grains has been claimed to be in the endosperm (Price and Parsons, 1979). Most oat cultivars have about 5–6% of oil and 55–60% of starch in the grain (Peterson and Welsh, 1995; Wood, 1997; Doehlert et al., 2001). Higher oil content is negatively correlated with starch content, and microscopic studies indicate that the oil content of the endosperm is higher in the high-oil varieties (Peterson and Wood, 1997). These data suggest that not only is a portion of the reduced carbon redirected from starch into oil synthesis in high-oil oat varieties, but also that these changes in metabolism occur within the endosperm cells. Identifying the regulatory enzymatic steps in the diversion of sugar into starch or oil in these cells will increase our understanding of the partitioning of carbon flow within the cereal seed.

A great number of analyses have been done regarding the total oil content and total fatty acid composition of grains from different oat varieties (Welch, 1995). However, the lipid content and composition in different parts of the oat grain have only been reported in a few works (De la Roche et al., 1977; Youngs et al., 1977; Price and Parsons, 1979; Sahasrabudhe, 1979), and none has reported data on lipid deposition during grain development. In this study, the lipid and fatty acid composition in different parts of the grain during seed development was characterized quantitatively and qualitatively in a medium-oil (6%) and a high-oil (10%) cultivar using thin-layer and
gas chromatography, and light and electron microscopy. The results give, for the first time, a comprehensive picture of lipid deposition in oat grains during grain development.

Materials and methods

Plant growth

Oat (A. sativa) seeds of cv. Freja and cv. Matilda (Svalöf Weihull AB, Svalöv, Sweden) were grown in parallel in a greenhouse with supplemented artificial light under a light/dark regime of 16/8 h. Seeds were harvested based on morphological criteria and dehulled prior to analysis: stage 1, grains were about 2 mm long with a fresh weight of approximately 7 mg; stage 2, grains were about 8 mm long, still green with a milky endosperm, and with a fresh weight of 40–45 mg; stage 3, grains were about 10 mm long, pale green with a jelly-like endosperm, and a fresh weight of 55–60 mg; and stage 4, mature grains with a seed weight of approximately 34 mg.

Grain analysis

From 10 to 90 grains (depending on the type of analysis and stage of development) from each developmental stage were pooled for the different chemical analyses. All analyses were done in quadruplicate. Diagrams are based on raw data that are all presented in the Supplementary data at *JXB* online. When calculating using analytical results from different parameters are done, the mean value of each parameter is used. Standard deviations are presented in figures where raw data have been used directly.

The embryo and scutellum were separated out from mature grains (i.e. stage 4) and the dry weight of these tissues as well as the dry weight of a similar number of intact grains was determined by drying them at 80 °C until a constant weight was achieved. It was not possible to dissect out the different tissue types from grains at stage 1, and the scutellum could not be separated intact at stages 2 and 3, and was thus analysed together with the embryo at those stages. The endosperm could not be separated from the grain, and data presented for the endosperm are calculated from data obtained for the whole grain from which the values (weights and contents) obtained for the embryo and scutellum were subtracted. Such data for the endosperm also comprise the aleurome layer, which constitutes a very low percentage of the total grain weight.

Protein, starch, and lipid analysis

Soluble protein content was determined by BCA protein assay reagent (Pierce) on supernatants of extracts of grains homogenized with an Ultra-Turrax® in TRIS-HCl buffer (pH 6.8) containing 1% SDS and 1 mM dithiothreitol (DTT), using bovine serum albumin (BSA) as a standard. Insoluble protein in the dried particulate residue from the extraction was determined by its nitrogen content with a Carlo Erba NA 1500 elementary analyser. The dry weight percentage of nitrogen was multiplied by a factor of 6.25 to give the percentage of insoluble protein. Only the sum of soluble and insoluble proteins is presented in this work. Starch content was determined by the amyloglucosidase/insoluble proteins is presented in this work. Starch content was determined by the amyloglucosidase method on crushed dried grains (McCleary et al., 1997).

Total lipids from oat grain tissues were extracted according to Bligh and Dyer (1959) by homogenizing in chloroform/methanol with an Ultra-Turrax®. Total fatty acid composition and content were determined by evaporating a fraction of the chloroform phase to dryness and methylating the fatty acid with sulphuric acid (2%) in methanol. Fatty acid methyl esters were extracted with hexane and separated by gas–liquid chromatography (GLC) using a WCOT fused silica 50 m x 0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack Inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard.

Neutral and polar lipids in the chloroform phase were separated by thin-layer chromatography using hexane/diethyl ether/acetic acid (70:30:1 by vol.) and chloroform/methanol/acetic acid/water (85:15:10:3.5 by vol.), respectively, using silica gel 60 plates (Merck, Darmstadt, Germany). The lipids were visualized by brief exposure to iodine vapour, and lipids were scraped from plates and heated under a stream of nitrogen to remove the iodine. Acyl groups were methylated in situ on the gel, and the fatty acid composition and content of individual lipids were determined by GLC as described above.

The oil content of grains was calculated as the total weight of the fatty acid as determined by GLC with addition of the weight of one glycerol molecule for three fatty acid molecules. Since 83–90% of lipids in the grains were triacylglycerols (Fig. 1a), this gives a good approximation of the oil content. It should be noted that protein and oil values using these methods will differ slightly from determinations using the standard Kjeldahl and Soxhlet methods.

Microscopic analysis

Immature grains from field-grown material of cv. Matilda were sampled at the mid-stage (stage 2) and late stage (stage 3) of development. The grains were fixed in glutaraldehyde and osmium tetroxide, dehydrated, embedded in epoxy resin, and sectioned for light microscopy and transmission electron microscopy. Sections for light microscopy were stained by methylene blue–azur A–safranin O (Warmke and Lee, 1976). For transmission electron microscopy, sections were stained by uranyl acetate and lead citrate, and studied in a Zeiss EM 10C microscope at an accelerating voltage of 60 kV.

Results

There were no significant differences in dry weight of the embryo, scutellum, and endosperm between the two cultivars. The endosperm made up 95% of the total grain weight, with the scutellum comprising about 3.5% and the embryo 1.2–1.6% of the weight (see Supplementary Table S1 at *JXB* online).

The majority of lipids are found in the endosperm and account for the higher oil content in grains of cv. Matilda

The amounts of acyl groups in polar and neutral lipids [of which >94% were triacylglycerols (TAGs), data not shown] in embryo with scutellum and in endosperm were determined in the two cultivars (Fig. 1a). The proportions between neutral and polar lipids were about the same in both tissue types within a cultivar, with Matilda having a higher proportion of neutral lipids (90%) than Freja (83–85%), and with the majority of both polar and neutral lipids found in the endosperm in both cultivars. The neutral lipid content of the embryo with scutellum in cv. Matilda was 30% higher than in cv. Freja, whereas the oil content in the endosperm was 99% higher.
endosperm contributed 95% of the higher oil content seen in grains of cv. Matilda compared with Freja.

Contents of polar lipids differ between different parts of the grain and between cultivars

The amounts of the four major polar lipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), digalactosyldiacylglycerol (DGDG), and monogalactosyldiacylglycerol (MGDG) were determined in embryo with scutellum and in whole grain of cv. Freja and Matilda (Fig. 1b). The amount of PC was similar in the embryo with scutellum in the two cultivars, whereas the PE was 20% less in cv. Matilda compared with Freja. Only a very small amount of DGDG and no MGDG were detected in the embryo and scutellum tissues. DGDG and PC were the dominant polar lipids of the whole grain, and were 28% and 23% higher, respectively, whereas MGDG was 34% less in cv. Matilda compared with Freja. PE content of the whole grain was 21% higher in cv. Matilda than in Freja.

Fatty acid composition differs between different parts of the grain and between cultivars

The total fatty acid composition of mature grains from both cultivars was determined in whole grain, embryo with scutellum, and embryo only (Fig. 2). The fatty acid composition differed between the two cultivars as well as between the tissue types. Cultivar Freja (Fig. 2a) had a lower percentage of oleic acid and a higher proportion of linoleic acid compared with cv. Matilda (Fig. 2b). The variation between the tissue types showed a similar trend in both cultivars. The embryo had higher levels of linoleic and linolenic acids than the whole grain, whereas embryo with scutellum had a higher palmitic acid content. Avenoleic acid, a 15-hydroxylated 18:2 fatty acid shown to be associated with DGDG (Hamberg et al., 1998), was not detected in the embryo or scutellum.

The majority of lipids are deposited before mid-stage of grain development

Oil, starch, and protein depositions were followed during grain development in cv. Freja and Matilda (Fig. 3). Both varieties accumulated most of the oil during the first half (stage 2) of grain development (84% and 79% of the final lipid content in cv. Freja and Matilda, respectively). At

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Fig. 1. Acyl contents of polar lipids (white bars) and neutral lipids (black bars) in different tissues of mature grains of cv. Freja and cv. Matilda (a). Content of various polar lipids (b) in embryo with scutellum (dashed bars) and whole mature grain (grey bars) of cv. Freja and cv. Matilda. PC, phosphatidylcholine; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol. Results are averages from four samples.

Fig. 2. Fatty acid composition of total lipids in different tissues of mature grain of cv. Freja (a) and cv. Matilda (b) in whole grain (grey bars), embryo with scutellum (dashed bars), and embryo (white bars). Fatty acids are 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:1, eicosa-11-enoic acid; 18:2-OH, avenoleic acid, D15-hydroxy-18:2. Results are averages ±SD from four samples.
this stage, only 52% and 38% of the starch had been deposited in cv. Freja and Matilda, respectively. Starch accumulation occurred at essentially the same rate in both varieties between mid-stage of development and maturity, resulting in 24% less starch in cv. Matilda compared with Freja. Protein accumulation proceeded throughout kernel development. Protein accumulation in cv. Matilda was 40% higher than in cv. Freja during the first half of grain development, whereas Freja had a higher rate of accumulation between mid-stage and maturity, resulting in the grains of Matilda having a 6% higher protein content compared with grains of Freja.

The timing of oil deposition differs between different parts of the grain and between cultivars

The accumulation of acyl groups was followed in embryo with scutellum and endosperm during grain development (Fig. 4a, b). The timing of fatty acid deposition was very different in the different tissues and also differed between the two varieties. The embryo with scutellum had accumulated 27% and 22% of their final fatty acid contents at stage 2 in cv. Freja and Matilda, respectively, and the amounts were equal in both varieties (Fig. 4a). The endosperm of cv. Freja and Matilda had, at this stage, already accumulated 94% and 85%, respectively, of their final fatty acid content (Fig. 4b). At maturity, the embryo with scutellum contained 14% and 10% of the total fatty acids in grains of cv. Freja and Matilda, respectively. Although 86% of the fatty acids in the mature grain of cv. Freja were found in the endosperm, the accumulation of fatty acids was over 2-fold higher in the embryo with scutellum than in the endosperm between stages 2 and 4 (Fig. 4c). In contrast to this, the endosperm in cv. Matilda accumulated 1.7 times more fatty acids than the embryo with scutellum during this period (Fig. 4c).

Changes in fatty acid composition during grain development

The total fatty acid profile of grains from the two cultivars was followed during the four stages of grain development (Fig. 5a, b). There was a drastic reduction in linoleic and linolenic acid in both cultivars between stage 1 and stage 2, after which the percentage of linoleic acid increased to maturity with a corresponding decrease in the relative amount of oleic acid. The levels of oleic acid were higher
and linoleic acid lower in cv. Matilda compared with Freja from stage 2 to maturity, resulting in a reverse ratio of oleic to linoleic acid in the mature grain of the two varieties. Over 90% of the accumulation of avenoleic acid occurred after the mid-stage of development.

By combining the data of fatty acid content from different parts of the grain, it can be calculated that there was a net increase of linoleic acid per endosperm between stages 2 and 4 in cv. Freja, exceeding the increase of total fatty acids in this tissue (Fig. 4c). The level of linoleic acid was always higher in PC than in TAG, but decreased in PC between stages 2 and 4, whereas it increased in TAG (see Supplementary Table S8 at JXB online). TAG was the only lipid class that showed an increased proportion of linoleic acid between stages 2 and 4, indicating that acyl groups in TAGs either undergo desaturation or are turned over.

Ultrastructural studies show abundant and fusing oil bodies in the endosperm

Although the percentage of lipids is considerably higher in the embryo and scutellum than in the endosperm, the main part of the lipids (86% and 90% in cv. Freja and Matilda, respectively) in the oat grain is clearly present in the endosperm. Therefore, it was of interest to document structural features of the different grain tissues at a mid-stage (approximately corresponding to stage 2) and a late stage (approximately corresponding to stage 3) of development. Light microscopy of sectioned grains at the mid-stage showed the aleurone cells and the adjacent endosperm cells in cv. Matilda (Fig. 6a). The aleurone layer contained abundant oil bodies and proteins, but no starch granules, whereas the subaleurone cells and the rest of the endosperm contained differentiatable starch granules, protein inclusions, and oil bodies. The staining of the oil bodies of the endosperm was darker than in the aleurone layer. Transmission electron microscopy showed that the oil bodies of the aleurone layer (Fig. 6b) and the embryo (Fig. 6c) occurred as individual uniform entities during both mid- and late stages of development. In contrast to this, the oil bodies of the endosperm tended to fuse with each other at the mid-stage of development, leading to their appearance as irregular shapes (Fig. 6d). At the late stage of development, individual or coalesced oil bodies were no longer discernible; instead the fusion of all oil bodies led to the presence of a continuous smear of oil in between the starch and protein components (Fig. 6e).

Discussion

The results from this study show that the majority of oat grain oil (86–90%) resides in the endosperm tissue, corroborating earlier findings (Price and Parsons, 1979). The lack of oil bodies within the endosperm of oat grains recently demonstrated by White et al. (2006) could either be due to loss of oil bodies during preparations of samples for microscopy studies, or due to variety-specific results. The endosperm including the aleurone layer was considered as one type of tissue in the present lipid analyses in
spite of the fact that the aleurone layer had a high concentration of lipids as evident from the microscopic studies. However, the aleurone layer consists of only 1–2 cell layers and could therefore only make up a low percentage of the total oil in the endosperm fraction.

**What is the function of the oil in the endosperm?**

The fact that most of the lipids are localized in the endosperm in oat raises interesting questions about the fate of these lipids during grain germination. Many seeds from dicotyledonous plants accumulate large amounts of lipids in the endosperm. These lipids are broken down through β-oxidation for energy production in the glyoxysomes, a special organelle that appears in the endosperm during germination. When the reserves in the endosperm are exhausted, the tissue goes through programmed cell death (Schmid et al., 1999). The endosperm of monocotyledons, on the other hand, undergoes programmed cell death when the grain matures (Young and Gallie, 2000) and thus has no capacity for β-oxidation during germination. The reserves in these endosperms are mobilized through amylases and proteases, mainly secreted from the aleurone layer, and the produced sugars and amino acids are taken up by the growing embryo. However, there are, to the best of our knowledge, no reports that lipases are secreted out into the endosperm and that released fatty acids are taken up in the embryo during grain germination. Oleosins are believed to stabilize and prevent fusion of oil bodies, thereby creating a large surface to volume area of oil droplets, facilitating rapid mobilization of the fatty acids by lipases during germination of oil seeds (Hsieh and Huang, 2004; Siloto et al., 2006). In line with this hypothesis, oil body fusions are seen in the maturing oat endosperm but not in the embryo, scutellum, and aleurone layer which, compared with the endosperm, are all living tissues. At a late stage of grain development, oil bodies are no longer visible as such in the endosperm in the ultrastructural studies, but the oil appears as a smear throughout the cells, possibly indicating a lack of oleosins within this tissue. It is pertinent to note that fusion of the oil bodies also occurs in the mesocarp of oil-rich fruits such as olives (Olea europaea) that lack oleosins (Ross et al., 1993). The oil in the mesocarp of these fruits does not serve as an energy source for the plant but as an attractant for fruit predators which thereby help to disperse the seeds. Fusion of oil bodies has also been shown to occur during rehydration in the desiccation-tolerant and oleosin-lacking oil seeds Azadirachta indica, Theobroma cacao, and Quercus rubra (Leprince et al., 1998).

**Higher oil content involves a switch in the utilization of glucose in the endosperm**

Two cultivars of oat, cv. Matilda and Freja, with different oil content were investigated. These cultivars were chosen since their grain weights do not differ significantly, which is important in order to carry out quantitative comparisons of the differences in storage products. The starch content was lower in cv. Matilda than Freja (55% versus 72%) which was compensated for by an increase of oil concentration (10% versus 5.9%) and an increase in protein concentration (15.9% versus 15.0%). That the oil content is strongly negatively correlated with starch content and positively correlated with protein content has been reported earlier (Peterson and Wood, 1997). If the energy values of these three storage products are calculated for the whole grain (oil having 2.25 times more energy than starch and proteins), cv. Freja and Matilda have the same value, demonstrating that total energy laid down in the grain does not have to be compromised by the high oil content.

The increase in oil content seen in cv. Matilda compared with Freja was up to 95% due to an increase in endosperm oil, indicating that the major difference between the varieties is a higher proportion of photosynthetic being channelled to oil instead of starch in cv. Matilda endosperm cells. A major quantitative trait locus (QTL) for oil content has been linked to an acetyl-CoA carboxylase (ACC) gene (Kianian et al., 1999). ACC catalysis is the first committed step in fatty acid biosynthesis. However, this step is far downstream of the utilization of the glucose for starch synthesis, which makes it unlikely to be the main determining enzyme for the switch from starch to oil synthesis in the endosperm cells.

**The endosperm switches glucose utilization to favour starch synthesis over oil synthesis after the mid-stage of grain development**

An unexpected finding was that the majority (79–84%) of the total oil was deposited during the first half of grain development when the endosperm was still liquid, whereas both protein and starch deposition proceeded with the same rate to a late stage of development. Actually, the rate of starch accumulation only differed during the first half of grain development between cv. Freja and Matilda. While the lipid deposition in the endosperm of cv. Freja practically ceased after the mid-stage (6% increase), it proceeded to grain maturity in embryo with scutellum (274% increase). In contrast, the oil deposition in the endosperm increased about 17% further between mid-stage and grain maturity in cv. Matilda. Thus, cv. Matilda had both a higher rate of oil deposition in the endosperm than cv. Freja and the deposition continued for a longer period, albeit at a much lower rate after the mid-stage of development.

**The changes in the ratios of oleate to linoleate indicate that the acyl groups in triacylglycerol are turned over**

The higher proportion of oleic acid and lower percentage of linoleic acid in cv. Matilda compared with Freja is in...
Supplemental material is available at JXB online.

The function of the endosperm oil in the germinating grain. Cereals should include TAG turnover in the endosperm and high-oil cultivar. Future studies on the lipid metabolism of confined to an increase in oil content of the endosperm in a medium-oil and a high-oil cultivar was nearly totally the endosperm. The differences in oil content between that the major part of the lipids are accumulated as TAGs in lipids in oat grain takes place during grain development and a highly temporal accumulation of protein and starch, a highly temporal accumulation of TAGs turnover in the endosperm and the function of the endosperm oil in the germinating grain.

Concluding remarks

In summary, this work shows that, unlike the accumulation of protein and starch, a highly temporal accumulation of lipids in oat grain takes place during grain development and that the major part of the lipids are accumulated as TAGs in the endosperm. The differences in oil content between a medium-oil and a high-oil cultivar was nearly totally confined to an increase in oil content of the endosperm in the high-oil cultivar. Future studies on the lipid metabolism of cereals should include TAG turnover in the endosperm and the function of the endosperm oil in the germinating grain.

Supplementary data

Supplemental material is available at JXB online.

Figure S1. Photos of oat grains (cv. Freja) at stages 1, 2, 3, and 4 of development.

Figure S2. Relative amount of oleic (black bars) and linoleic acid (white bars) in total polar lipids (a), phosphatidylcholine (PC) (b), and triacylglycerols (TAGs) (c) in whole grain during development in cv. Freja. Results are averages±stddev from 4 samples.

Table S1. Fresh weight of embryo, embryo with scutellum and whole grain and dry weight of whole grain during different stages of development.

Table S2. Polar and neutral lipid contents of embryo with scutellum and whole mature grain expressed as nmol fatty acids per mg fresh weight.

Table S3. Fatty acid composition of mature embryo, embryo with scutellum and whole grain.

Table S4. Fatty acid, protein and starch contents per mg fresh weight in whole grain during different stages of development.

Table S5. Fatty acid content per mg fresh weight in embryos with scutellum during different stages of development.

Table S6. Fatty acid composition of embryo with scutellum during different stages of development.

Table S7. Fatty acid composition of whole grain during different stages of development.

Table S8. Relative amount of oleic and linolenic acid in total polar lipids, phosphatidylcholine and triacylglycerols in whole grain during development in var. Freja.

Table S9. Content of various polar lipids in whole grains of cv. Freja and cv. Matilda during grain development.

Table S10. Content of various polar lipids in embryo and in scutellum of cv. Freja and cv. Matilda at development stage 4.

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References


nine cycles of recurrent selection for increased oil content in oat.


