Developmental Changes in Fatty Acid Composition of Oil in Kernel Fractions of Corn (*Zea mays* L.)[1]

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Abstract

Gas liquid chromatography was used to determine the change in fatty acid composition of oil from three kernel fractions (pericarp, endosperm and germ) during kernel maturation of four inbred lines of corn. Inbred lines were sib-pollinated, and sampling of ears began six days after pollination (DAP) and continued at three day intervals until 33 DAP and then at weekly intervals until 54 DAP. Proportion of palmitic acid in the pericarp oil rapidly decreased between 6 and 12 DAP while oleic and linoleic acids increased during the same period. Changes in fatty acid composition of oil from the endosperm during kernel maturation were erratic and no consistent trends were evident. In the germ oil, palmitic and linolenic acid proportions decreased during kernel maturation, while oleic acid decreased and linoleic acid increased during kernel maturation for three of the four inbred lines. By about 24 to 27 DAP, the fatty acid composition of oil in the mature kernel was established. Since kernel fractions are of different genetic origin, a study of developmental changes in lipid classes or in fatty acid composition of oil should be limited within kernel fractions that have a similar genetic constitution.

Introduction

Corn is a major crop and, despite its low oil content, large quantities of oil are available as a by-product from the wet-milling of corn for starch. Until recently, little information was available on the developmental changes in oil composition of the maturing corn kernel. Weber (1) studied whole kernel lipids and showed that the most rapid change in fatty acid composition occurred between 10 and 30 days after pollination (DAP). In the triglycerides, the percentages of palmitic, linoleic and linolenic acids decreased and oleic acid increased until about 45 DAP when no further change was observed. In the polar lipids, the percentages of palmitic and linolenic acids decreased and oleic acid increased during kernel maturation. The change in the polar lipids occurred over a longer period of seed maturation as compared with the triglycerides. Linoleic acid per cent of the polar lipids did not change until about 45 DAP when the relative proportion of linoleic acid decreased until kernel maturity. Weber (1) also showed that the total oil and the amount of each fatty acid increased (except for the inbred line K6) during kernel maturation. Curtis et al. (2) compared the germ oil composition of one immature stage (24 DAP) with the oil composition of mature kernels. Proportion of palmitic acid was less in the mature kernels. Three of the five strains studied showed an increased proportion of linoleic acid and a decreased proportion of oleic acid at maturity. No data were given for linolenic acid.

Earlier studies (3,4) of developmental changes in corn oil composition were made by measuring the iodine number of the oil during kernel maturation. Brimhall and Sprague (3) sampled corn, beginning at 20 DAP, and found no change in iodine number for two inbred lines and one single cross. Evans (4) sampled a corn hybrid and showed a steady increase in iodine number from 15 to 36 days after silking. Iodine values remained constant after 36 days from the silking date.

The mature corn kernel is made up of three main parts which have different hereditary makeup. The genetic composition of the pericarp is similar to the maternal plant that produced the seed; the hereditary makeup of the endosperm is two thirds maternal and one third paternal; and the germ (scutellum and embryo axis) is a true diploid or equal inheritance from the two parents. A large proportion of the very immature kernel is pericarp. Soon after fertilization, the endosperm develops earlier and more rapidly than the germ. The germ is very small and hardly visible until about 12 to 15 days after fertilization. Therefore, sampling of the whole kernel to study developmental changes would include different fractions of the kernel as significant variables. Early samples would represent lipids from the pericarp and endosperm while the mature samples would represent lipids mainly from the germ. The lipids in endosperm and germ fractions of the corn kernel have been shown to differ in fatty acid composition (5). No data are available on the developmental changes in oil composition in the different kernel fractions of corn. This study was initiated to determine changes in oil composition in the pericarp, endosperm and germ fractions of the kernel during maturation.

A number of studies have been reported concerning the developmental changes in oil composition and content in the oilseed crops. These studies have been made on soybean, sunflower, safflower, peanut, flax, crambe, rape and castorbean. In general, rapid changes in oil composition and content occurred during the early stages of seed development. Worthington (6) studied different tissues of the developing peanut seed and found differences in oil content and oil composition to be related to tissue type as well as stage of seed development. A literature review of the oilseed crops is not given herein and the reader is referred elsewhere (1,6) for a complete citation and review concerning developmental changes in oil composition and content of oilseed crops.

Experimental Procedures

Corn Inbred Lines

Four inbred lines (Pa36, HO-11, 1–274 and T×341) known to differ in fatty acid composition of oil were selected for study. Pa36 was developed and released by the Pennsylvania Agricultural Experiment Station and is characterized by its high linoleic acid percentage (66–67%) of the germ oil at maturity; HO-11 was developed at Virginia Polytechnic Institute and is low in palmitic acid (8–9%) of the germ oil at

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maturity; 1–274 was developed at the Coastal Plain Experiment Station, Tifton, Ga., and is high (20–21%) in palmitic acid proportion of oil at maturity; T×341 was developed by the Texas Agricultural Experiment Station (College Station) and is high in stearic acid (4–5%) and oleic acid (47–48%) and low in linoleic acid (33–34%). All inbred lines were of the yellow endosperm type except 1–274, which was white. Inbred lines were planted on May 8, 1968. Ear shoots were bagged before silking and sib-pollinations of each inbred were all made on the same day as follows: Pa36 (July 7), HO-11 (July 13), 1–274 (July 23), and T×341 (July 23).

Sampling Technique and Sample Preparation

Four well pollinated ears were collected from each inbred line beginning 6 DAP, at three day intervals until 33 DAP, and then at weekly intervals until 54 DAP. One additional sample (about 90 DAP) was collected in late October after reaching minimum grain moisture content in the field. A total of 14 samples was collected for each inbred line. All ears were collected at the same time of day (usually between 8 and 9 A.M.) and stored at 0 C until further processing.

Due to the small kernel size, no separation was made at 6 DAP; whole kernel samples were analyzed and considered as pericarp fractions. This was also true for the 9 DAP samples except from Pa36. At 9 DAP, Pa36 was separated into pericarp and endosperm-germ. The first germ fractions were obtained at 12 DAP from Pa36 and 1–274 and at 15 DAP from HO-11 and T×341. The pericarp was not separated from the endosperm for the last several sampling dates. Kernels were hand-separated into different fractions and air-dried for one day before extraction. Previous gas liquid chromatography (GLC) analyses showed no difference in oil composition of dried and undried fractions. Kernels were removed from the middle portion of the ear to eliminate possible kernel position effects (7). Except for some pericarp samples and some very early germ samples, four oil samples (replicates) were obtained from each kernel fraction of each inbred line.

Kernel fractions were extracted overnight in a 2:1 mixture of methanol-petroleum ether (Skellysolve F). The kernel fractions were left in 25 ml volumetric flasks during preparation of the methyl esters by the methanol-sulfuric acid procedure. Handling of oil samples was similar to that previously described by Jellum and Worthington (8).

Analysis by GLC

Analyses were made with a Varian Aerograph Model 1200–2 gas chromatograph (flame ionization detector) using an Elhygen Model E-150 hydrogen generator. Peak areas were measured with an Infotronics Model CRS-11HSB digital integrator. A Honeywell ElectroniK 18 recorder was used to monitor column performance. Methyl esters were separated on a 1.83 m by 6.35 mm copper column packed with 10% (by weight) of stabilized diethylene glycol succinate (DEGS) on 80/100 mesh Aeropak 30 (Varian Aerograph) solid support. Injection port, column oven and detector were operated at 270, 220 and 300 C, respectively. Retention time for linolenic acid was 6 min. Helium was used as the carrier gas. National Heart Institute standards KA, KB and KD were analyzed with less than 5% error for major and minor components.

Statistical Analysis of Data

Data for each kernel fraction of each inbred line were subjected to analysis of variance. Combined analyses were not performed since inbred lines and kernel fractions were obviously different from each other. Differences among means of sampling dates were determined by Duncan's Multiple Range Test at the 5% level of significance.

Results and Discussion

Pericarp

The immature kernel at 6 DAP consists mainly of the pericarp fraction and a small amount of endosperm. As the kernel matures, the pericarp decreases in percentage of the total kernel. Changes in fatty acid composition of the oil in the pericarp during kernel maturation of the four inbred lines is shown in Figure 1. The most rapid and significant changes in oil composition occurred in the early development

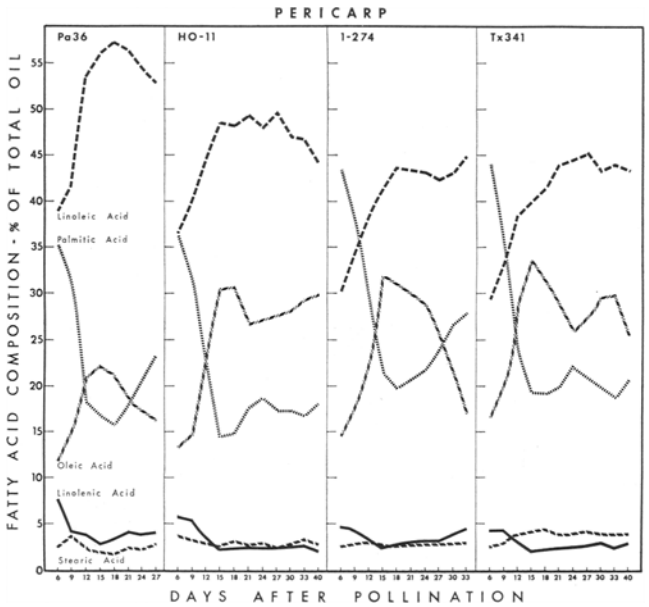

FIG. 1. Fatty acid composition of oil extracted from the pericarp fraction during kernel maturation of four inbred lines of corn.

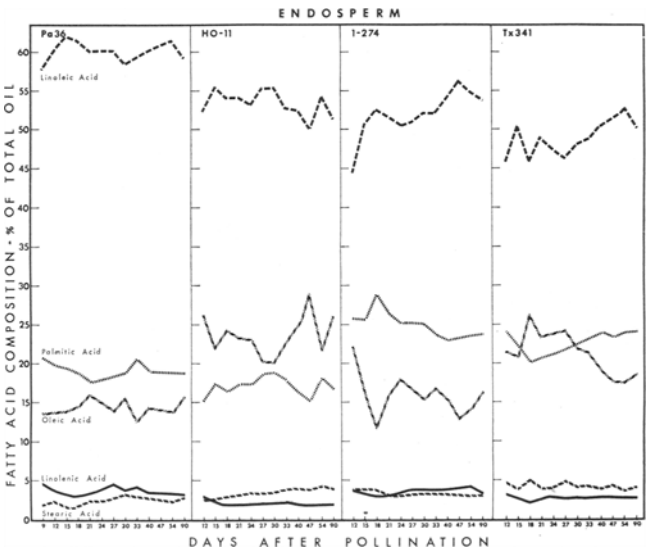

FIG. 2. Fatty acid composition of oil extracted from the endosperm fraction during kernel maturation of four inbred lines of corn.

period before 12 to 15 DAP. In all inbred lines, palmitic acid proportion of the oil decreased very rapidly from 6 to 12 DAP and reached a low level at 18 DAP and then increased with maturity (except T×341). Oleic and linoleic acids rapidly increased from 6 to 12 DAP and reached high values at about 15 to 18 DAP. Oleic acid reached a high percentage at 15 DAP for Pa36 and 1–274 and then decreased for each sampling date until 27 and 33 DAP, respectively. Stearic acid did not show any similar consistent trend for all inbred lines. Linolenic acid decreased during 6 to 15 DAP and then showed an upward trend as the kernel matured.

Endosperm

The endosperm was first separated at 9 DAP for Pa36 and at 12 DAP for the other inbred lines. Changes in oil composition of the endosperm during kernel maturation are shown in Figure 2. Fatty acid composition fluctuated for Pa36 and HO-11 during kernel maturation and no definite trends were evident. Although erratic, oleic acid tended to decrease and linoleic to increase in percentage during maturation of 1–274 and T×341. Opposite trends were shown for palmitic acid, decreasing in 1–274 and increasing in T×341.

Germ

The germ is very small and hardly visible at 12 DAP, but it rapidly increases in size between 15 and 30 DAP. Other studies (2,9) have reported the third and fourth weeks after pollination as the most active period of total oil synthesis. However, the greatest change in oil composition occurs before 21 DAP (Fig. 3) or before there is a rapid increase in total oil. Except for T×341, the major changes in oil composition of the germ occur during the period before 21 DAP. Oil composition of T×341 changed over a longer period of time and changes were evident until maturity. Palmitic and linolenic acid percentages decreased during maturation for all four inbred lines. Oleic acid percentage decreased during maturation for Pa36, HO-11 and 1–274, but increased in T×341. Linoleic acid showed opposite trends (increased in all inbreds except T×341) as compared to oleic acid (Fig. 3). Weber (1) showed oleic acid to increase in lipids from the whole kernel which is similar to the results for T×341 but not the other three inbred lines in this study.

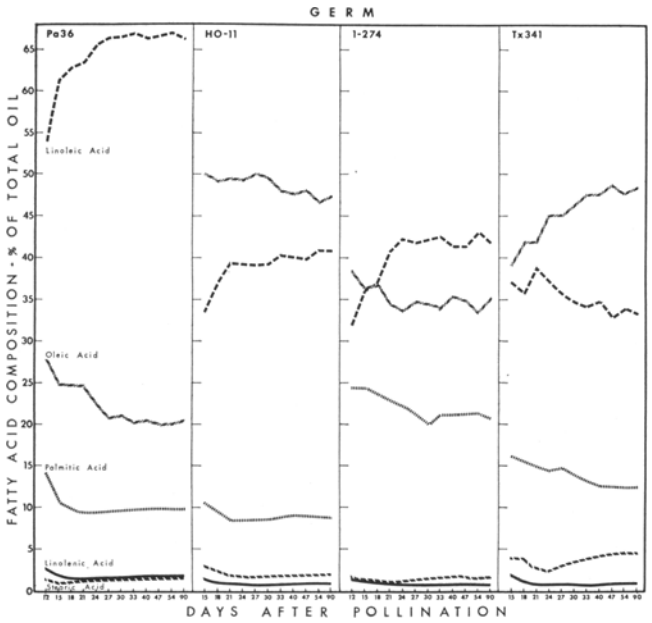

FIG. 3. Fatty acid composition of oil extracted from the germ fraction during kernel maturation of four inbred lines of corn.

Minor Component Fatty Acids

Minor component fatty acids were not mentioned in previous studies (1,2) of corn oil. Chromatograms, which illustrate changes in major and minor component fatty acids of inbred line 1–274, are shown in Figure 4. These chromatograms were obtained with a 2.44 m by 6.35 mm copper column packed with 10% DEGS on Chromosorb W. Retention time for linolenic acid was 10 min instead of 6 min for the column used for obtaining the statistically analyzed data reported herein. Differences in number and proportion of minor component fatty acids among kernel fractions as well as between immature and mature stages of kernel development are evident in Figure 4. Similar results as illustrated in Figure 4 were observed for the other three inbred lines.

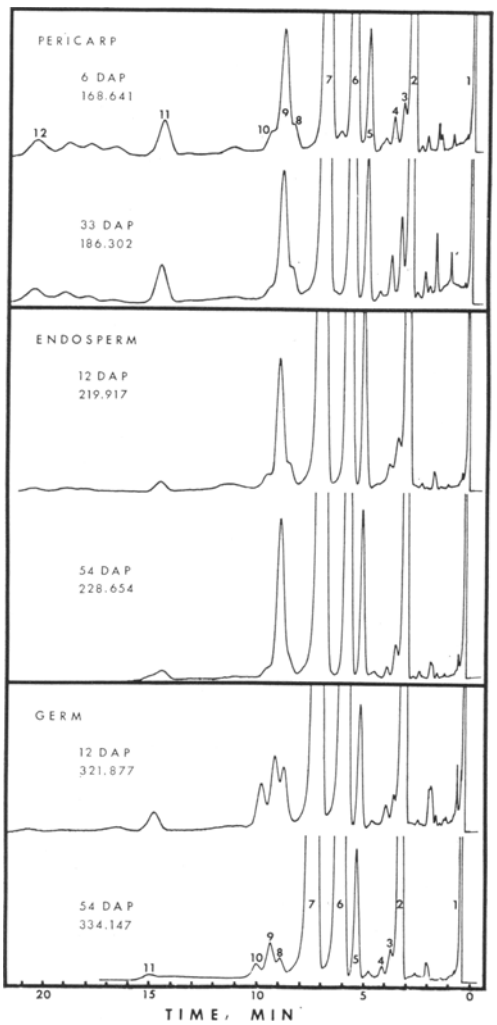

FIG. 4. Chromatograms of methyl ester samples from oil of immature and mature kernel fractions of the inbred line 1–274. The number below the maturity stage is the total digital integrator count for comparing the size of oil sample chromatographed. Peak identification: 1, solvent-Skellysolve F; 2, palmitic; 3, palmitoleic; 4, margaric; 5, stearic; 6, oleic; 7, linoleic; 8, arachidic; 9, linolenic; 10, eicosenoic; 11, behenic; and 12, lignoceric acid.

Statistical Analyses

Except for some pericarp and early germ samples, four oil samples were extracted and chromatographed from each kernel fraction and each maturity stage. Fatty acid composition of the oil samples from each fraction and maturity stage were very uniform with coefficients of variation ranging from 1.7% to 4.8% for palmitic, 2.8% to 7.4% for stearic, 1.1% to 7.0% for oleic, 0.7% to 2.5% for linoleic, and 5.6% to 11.6% for linolenic acid. Therefore, small differences in oil composition between maturity stages as shown in Figures 1, 2 and 3 were statistically significant. Differences between means of approximately 0.5% to 1.5% for palmitic acid, 0.2% to 0.4% for stearic acid, 1% to 2% for oleic and linoleic acids, and 0.2% to 0.6% for linolenic acid were statistically significant. Numerical values and significant differences between means as determined by Duncan's Multiple Range Test for the results in Figures 1, 2 and 3 are available by request from the author.

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