Lipids of Maturing Grain of Corn (*Zea mays* L.): I. Changes in Lipid Classes and Fatty Acid Composition[1]

EVELYN J. WEBER, Cereal Crops Research Branch, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, University of Illinois, Urbana, Illinois 61801

Abstract

The total lipids of the grain from three strains of corn were compared throughout the growing season. The Illinois High Oil stock and the two inbreds, H51 and K6, represented high, intermediate and low oil-producing lines. In all three strains lipid synthesis was most active between 15 and 45 days after pollination. The lipids were extracted from the grain with a mixture of chloroform, methanol and water and were separated into classes by silicic acid and thin layer chromatography. Triglycerides constituted 10–17% of the total lipids at 10 days after pollination and increased to 75–92% at 75 days. Polar lipids at 10 days represented 70–72% and at 75 days 4–21%. Fatty acid compositions of the triglycerides and polar lipids changed as the grain matured, but the fatty acids of the polar lipids were more saturated than those of the triglycerides throughout the sampling periods. The major polar lipids were digalactosyl diglyceride, monogalactosyl diglyceride, phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine.

Introduction

To study biosynthesis of fatty acids in a seed, one must know the period when this seed is most actively synthesizing lipids. Changes in fatty acid composition and lipid content have been observed in the maturing seed of barley (1), castor bean (2), crambe (3), flax (4–7), oats (1), rape (3,8), safflower (4,9), sunflower (10) and wheat (1,11). Rapid synthesis of the fatty acids occurred only during a brief period early in the development of these commercial, oil-bearing seeds. In flax and safflower over 95% of the oil was synthesized between 10 and 40 days after fertilization (4). Ricinoleic acid did not appear in castor beans until the seed was 12 days old but represented 90% of the fatty acids when the beans were 36 days old (2).

The utilization of the fatty acids by the developing seed can be determined by examining the changes in lipid classes. McKillican and Sims (12,13) have separated the lipid classes of flax, safflower, rape and crambe. Daftary and Pomeranz (14) examined the lipid classes of maturing wheat.

Although corn is a major crop and an important source of commercial oil, very little information is available on lipid synthesis in the developing corn kernel. In 1941, Evans (15) determined the iodine values of ether extracts from a maturing field corn hybrid. Brimhall and Sprague (16) also followed changes in unsaturation of the fatty acids from two inbreds and a single cross by iodine number. In the present investigation gas, column and thin layer chromatography were used to determine changes in lipid classes and fatty acid composition in maturing corn.

[1] Presented in part at the AOCS-AACC Joint Meeting, Washington, D.C., April 1968.

Grain was collected from the Illinois High Oil (IHO) stock and two inbreds, H51 and K6. These particular lines of corn were chosen, because they represented a range of oil contents. Illinois High Oil has undergone 66 generations of mass selection for high oil content (17); K6 has a low oil content and H51 is intermediate. These three strains also differ in fatty acid composition. IHO has the highest proportion of oleic acid and K6 the lowest. Conversely, linoleic acid is highest in K6 and lowest in IHO. The changes in fatty acid composition were followed both within and between lipid classes.

Experimental Procedures

Corn Samples

The corn was grown during the summer of 1966 on the Agronomy farm of the University of Illinois, Urbana, Illinois. Samples of IHO, H51 and K6 were collected at intervals after the dates of hand-pollination.

The husks and silks were stripped from the immature corn in the field. The ears were packed with dry ice in insulated containers and taken to the laboratory where the frozen kernels were removed intact from the ears with a dissecting knife. Kernel weight and moisture were determined by weighing 100 kernels before and after drying in a vacuum oven at 60 C for 24 hr.

Lipid Extraction

Frozen kernels from several ears of the same line were pooled for each sample. Total lipids were extracted by homogenizing the grain in a mixture of chloroform, methanol and water by the procedure of Bligh and Dyer (18). The immature corn was homogenized in a Virtis 45 tissue grinder. Samples collected later than 60 days after pollination were too hard to break up with the homogenizer. This mature corn was ground in a Spex mill before extraction.

The lipid extracts were evaporated nearly to dryness under vacuum. After the lipids had been redissolved in a known volume of chloroform-methanol-water (86:14:1) and filtered, two 1 ml aliquots were dried to constant weight for the determination of the percentage of lipid.

All the lipid samples were stored in solution at −15 C under nitrogen and in the dark. Extraction of the grain immediately after harvesting gave the best results. If the samples were lyophilized, stored and then extracted, larger amounts of free fatty acids were found.

Separation of Lipid Classes by Column and Thin Layer Chromatography

The total lipid extracts were fractionated on silicic acid columns. The silicic acid (Mallinckrodt, 100 mesh, especially prepared for chromatographic analysis) was washed with water and methanol to remove fines and impurities. It was activated at 120 C overnight and again for 1 hr immediately before the column was prepared.

TABLE I

Lipid Classes

Strain	DAP[a]	100 kernels Wet wt, g	100 kernels Dry wt, g	Oil % dry wt	Hydrocarbons sterol esters[b]	Triglycerides[b]	Free fatty acids[b]	Sterols[b]	Partial glycerides[b]	Polar lipids[b]
IHO	10	8.3	1.1	3.0	4.7	10.1	0.5	6.8	7.1	70.8
	15	15.6	2.5	5.6	4.2	41.1	0.5	5.0	4.1	45.1
	30	26.7	11.5	10.9	1.6	78.4	1.4	4.3	4.7	9.6
	45	31.6	18.0	13.7	2.0	84.0	0.5	3.2	4.3	6.0
	60	30.6	19.0	13.8	1.5	88.1	0.4	2.5	3.0	4.5
	75	33.4	23.4	13.4	1.3	92.0	0.4	1.5	0.9	3.9
	90	31.8	23.8	13.8	1.0	92.4	0.3	1.3	1.1	3.9
H51	10	6.6	0.8	3.3	3.9	13.0	0.4	6.8	6.2	69.7
	20	9.7	1.5	3.9	5.3	24.0	1.1	5.6	4.7	59.3
	30	30.5	11.9	5.6	2.5	68.8	1.0	3.7	5.3	18.7
	45	28.7	15.5	6.1	2.9	75.4	1.3	4.5	5.9	10.0
	60	19.3	16.0	5.3	2.9	79.0	1.0	4.5	3.9	8.7
	75	22.4	19.9	5.7	2.9	80.8	0.9	5.1	3.4	6.9
	85	23.3	21.9	5.2	2.8	80.7	0.9	4.6	2.9	8.1
K6	10	6.0	0.7	3.4	4.9	17.3	0.4	3.0	2.0	72.4
	15	10.0	1.7	3.6	3.9	27.8	0.3	2.0	2.0	63.1
	30	26.9	11.0	2.9	3.5	56.3	....	4.3	2.8	33.1
	45	31.1	17.7	3.9	3.4	74.4	0.7	5.2	2.4	13.9
	60	30.7	20.3	2.4	3.4	75.8	1.1	4.6	2.1	13.0
	75	21.2	16.5	2.6	4.5	74.9	0.4	5.5	2.7	12.0

[a] DAP, days after pollination.
[b] Relative weight per cent.

For each column 20 g of silicic acid were washed with 240 ml of chloroform-methanol (7:1 v/v), 120 ml chloroform-methanol (15:1 v/v), and 160 ml chloroform. A slurry of the silicic acid in chloroform was poured into the column (15 mm i.d.). After the column was dehydrated with 100 ml of diethyl ether and 300 ml of 4% diethyl ether in petroleum ether (bp 60–68 C), 300 mg of lipid were applied.

The solvent systems used to elute the column were similar to those described by Hirsch and Ahrens (19). In a typical fractionation 60 ml of 4% diethyl ether in petroleum ether eluted the hydrocarbons and sterol esters; 600 ml of the same solvent system removed the triglycerides and an additional 50 ml the free fatty acids. Sterols were eluted with 250 ml of chloroform and partial glycerides and carotenoids with an additional 350 ml of chloroform. The polar lipids, glycolipids and phospholipids, were removed from the column with 300 ml of methanol. The lipid classes were checked and purified further, if necessary, by thin layer chromatography (TLC). These plates were developed in petroleum ether-diethyl ether-acetic acid (80:20:1) (20).

Gas Liquid Chromatography

Methyl esters of the fatty acids were prepared by heating 10 mg of the lipid with 1 ml of 5% sulfuric acid in methanol at 102 C for 2 hr. For the transmethylation the lipid samples were sealed under nitrogen gas in screw-capped vials with Teflon tape and cap liners. The methyl esters were removed from the acid solution by three extractions with 10 ml portions of petroleum ether. The combined ether extracts were washed three times with equal volumes of water to remove any residual traces of acid and dried over sodium sulfate.

The methyl esters in a solution of petroleum ether were injected into a gas chromatograph (F & M Scientific Corporation Model 400) equipped with a flame ionization detector. The ¼ in. × 6 ft glass column was packed with 12% stabilized diethylene glycol succinate polyester on Anakrom ABS, 70/80 mesh (Analabs, Inc.). The carrier gas was helium at 40 psig, and its flow rate was 40 ml/min. The column temperature was maintained at 180 C. The percentages of the fatty acids were calculated by determining the areas of the peaks with the aid of a Disc integrator. Analysis of fatty acid standards KB and KD (Applied Science Laboratories, Inc.) of the National Heart Institute types (21) agreed with the stated compositions with a relative error of less than 2.5% for the major components (> 10% of total mixture) and less than 5% for minor components (< 10% of total mixture).

TABLE II

Accumulation of Fatty Acids in Developing Kernels[a]

Strain	DAP[b]	Total lipids, mg	Triglycerides, mg	Individual fatty acids, mg 16:0	18:0	18:1	18:2	18:3
IHO	10	33.2	3.4	0.6	0.1	0.4	1.8	0.5
	15	140.4	57.7	8.1	1.0	20.6	26.6	1.4
	30	1254.9	983.8	136.7	12.8	397.5	433.9	2.9
	45	2464.8	2070.4	265.0	35.2	728.8	1029.0	12.4
	60	2631.6	2318.4	329.2	39.4	816.1	1124.4	9.3
	75	3139.6	2888.4	332.2	37.5	924.3	1565.5	28.9
	90	3275.4	3026.5	381.3	63.5	1092.6	1470.9	18.2
H51	10	26.4	3.4	0.7	0.1	0.6	1.7	0.3
	20	58.2	14.0	2.8	0.3	3.3	7.0	0.6
	30	671.0	461.6	88.2	7.8	146.3	214.6	4.6
	45	947.1	714.1	127.8	10.7	226.4	342.1	7.1
	60	849.2	670.9	118.8	11.4	206.6	326.1	8.0
	75	1142.4	923.1	160.6	12.9	294.5	445.9	9.2
	85	1141.7	921.4	162.2	13.8	286.6	444.1	14.7
K6	10	24.0	4.2	0.6	0.1	0.4	2.5	0.6
	15	60.0	16.7	2.5	0.2	3.3	9.6	1.1
	30	322.8	181.7	23.1	1.8	51.1	102.1	3.6
	45	684.2	509.0	59.5	6.1	124.7	309.0	9.7
	60	491.2	372.3	43.6	5.2	79.7	236.0	7.8
	75	424.0	317.6	39.7	3.5	67.3	200.4	6.7

[a] Wt/100 kernels.
[b] DAP, days after pollination.

TABLE III
Fatty Acid Composition
Triglycerides

Strain	DAP[a]	Area, %				
		16:0	18:0	18:1	18:2	18:3
IHO	10	19.1	2.1	10.9	51.7	16.2
	15	14.0	1.7	35.7	46.2	2.4
	30	13.9	1.3	40.4	44.1	0.3
	45	12.8	1.7	35.2	49.7	0.6
	60	14.2	1.7	35.2	48.5	0.4
	75	11.5	1.3	32.0	54.2	1.0
	90	12.6	2.1	36.1	48.6	0.6
H51	10	20.4	2.0	19.6	50.0	8.0
	20	20.3	2.1	23.7	49.6	4.3
	30	19.1	1.7	31.7	46.5	1.0
	45	17.9	1.5	31.7	47.9	1.0
	60	17.7	1.7	30.8	48.6	1.2
	75	17.4	1.4	31.9	48.3	1.0
	85	17.6	1.5	31.1	48.2	1.6
K6	10	15.0	1.7	10.5	58.2	14.6
	15	14.9	1.2	19.8	57.6	6.5
	30	12.7	1.0	28.1	56.2	2.0
	45	11.7	1.2	24.5	60.7	1.9
	60	11.7	1.4	21.4	63.4	2.1
	75	12.5	1.1	21.2	63.1	2.1

[a] DAP, days after pollination.

TABLE IV
Fatty Acid Composition
Polar Lipids

Strain	DAP[a]	Area, %				
		16:0	18:0	18:1	18:2	18:3
IHO	10	27.0	1.5	8.7	49.8	13.0
	15	24.5	1.3	8.5	58.0	7.7
	30	22.1	0.9	12.1	59.7	5.2
	45	20.6	1.2	12.9	60.9	4.4
	60	22.7	1.8	15.9	55.2	4.4
	75	21.4	1.4	23.3	50.5	3.4
	90	21.5	1.3	28.4	46.9	1.9
H51	10	25.4	1.3	5.4	57.2	10.7
	20	24.8	0.9	5.2	60.4	8.7
	30	23.8	1.2	11.4	58.9	4.7
	45	20.0	0.8	12.0	61.0	6.2
	60	22.4	1.2	26.6	46.7	3.1
	75	20.3	1.4	28.3	47.6	2.4
	85	21.2	1.5	27.6	46.9	2.8
K6	10	27.4	1.0	8.7	52.1	10.8
	15	25.5	1.5	9.5	55.2	8.3
	30	24.5	1.3	9.2	58.6	6.4
	45	20.4	1.2	10.8	59.4	8.2
	60	20.2	1.7	11.5	58.9	7.7
	75	24.8	1.5	18.8	51.6	3.3

[a] DAP, days after pollination.

Results and Discussion

Changes in Weights of Kernels

The wet weights of the developing corn kernels (Table I) increased rapidly for 45 days after pollination and then began to level off or decrease. The dry weight gain was greatest from 15 to 45 days after pollination in all three strains. After 45 days the dry matter accumulation was much slower.

Changes in Total Lipid Content

The total lipids extracted from the corn are given as per cent of the dry weight of the kernels in Table I and as grams per 100 kernels in Table II. The rate of lipid synthesis was greatest between 15 and 45 days after pollination in all three strains. This corresponded with the dry weight accumulations. After 45 days the percentage of oil in H51 did not change. In K6, the low oil variety, it decreased. Although the percentage of oil in IHO remained constant after 45 days, the total weight of oil (Table II) increased.

More oil was synthesized by IHO than by H51 or K6 at all sampling dates except 10 days after pollination. ^{14}C-Acetate studies with 10-day-old tissue showed no difference in the rates of incorporation into fatty acids between IHO and H51. With 35-day-old tissue the incorporation by IHO was 1.2 times that of H51.

Leng (17) found that the dry weights of both the whole corn kernels and the germs of IHO increased linearly from 19 days to 49 days after pollination. The ratio of germ to whole kernel dry weight increased, and the germ oil percentage rose from 38% to 55%. As a result, the total oil in the whole kernel increased rapidly during this period. The major changes in the corn kernel brought about by selection for high oil content appear to be a larger ratio of germ to whole kernel and a higher percentage of oil in the germ.

Changes in Lipid Classes

Fractionation of the total lipid extracts on silicic acid columns revealed that as the grain matured, the relative proportions of the lipid classes changed (Table I). The sterol esters and hydrocarbons were minor components and decreased to even smaller fractions of the total lipids during development. At all collection dates the corn lipids contained only traces of free fatty acids. This contrasts with wheat (14), crambe (13) and rape (13) where the content of free fatty acids was relatively high in the immature seed but decreased as the seed developed.

Diglycerides were the dominant lipids but small amounts of monoglycerides and carotenoids were noted in the partial glycerides fractions. TLC indicated traces of other components in both the sterol and partial glycerides fractions. These spots were not identified.

The largest changes in the lipid classes occurred between the triglyceride and polar lipids fractions. In all three strains at 10 days after pollination the polar lipids fractions represented approximately 70% of the total lipids, but as the storage lipids, the triglycerides, were synthesized, the proportion of polar lipids declined rapidly. In only 30 days the polar lipids had fallen to 10% in Illinois High Oil. They reached the 10% level in H51 in 45 days. The low oil line, K6, had higher relative percentages of polar lipids throughout the collection periods. This was a reflection of the lower production of triglycerides by K6. At maturity only 75% of the total lipids of K6 were triglycerides. Illinois High Oil was characterized by 92% triglycerides; H51 had 81%.

Changes in Fatty Acid Composition of the Lipid Classes

The fatty acid compositions of the triglyceride fractions were analyzed by gas liquid chromatography. Table III shows the fatty acids expressed as per cent of total acids. From 10 to 45 days after pollination there were large changes in the fatty acid composition of the triglycerides. The percentages of palmitic, linoleic and linolenic fell and those of oleic acid increased. After 45 days the proportions of fatty acids in the triglycerides remained nearly constant.

If the weights of the individual fatty acids per 100 kernels are calculated (Table II), the largest increase in fatty acid synthesis in all three lines is found between 15 and 30 days after pollination. The absolute amounts of all five fatty acids increased as the grain developed, but oleic and linoleic were the major fatty acids accumulated in the triglycerides.

The fatty acid compositions of the polar lipids fractions are shown in Table IV. The fatty acids of the polar lipids had a higher percentage of palmitic

acid and consequently were more saturated than the triglycerides. The changes in the individual acids that occurred during maturation were similar to those of the triglycerides of the three lines. The relative percentages of palmitic and linolenic decreased and oleic increased. After 45 days the linoleic values fell. The resulting increase in saturation may indicate alterations in the membranes of the seed as it matures, for the polar lipids are vital components of these membranes.

The changes in fatty acid compositions of the polar lipids could reflect variations in the relative amounts of the individual lipids within the class. TLC revealed that digalactosyl diglyceride, monogalactosyl diglyceride, phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine are the principal polar lipids of corn. In mature wheat all of these lipids had characteristic fatty acid patterns (22). A study of the changes in the individual phospholipids and glycolipids of developing corn is in progress.

ACKNOWLEDGMENTS

Technical assistance by Lorraine Bollinger, aid in selecting and growing field samples by D. E. Alexander, Ian de la Roche and other members of the Plant Breeding Division, Agronomy Department, University of Illinois.

REFERENCES

1. Lindberg, P., E. Tanhuanpää, G. Nilsson and L. Waas, Acta. Agr. Scand. *14*, 297–306 (1964).
2. Canvin, D. T., Can. J. Biochem. Physiol. *41*, 1879–1885 (1963).
3. Sims, R. P. A., Can. J. Plant Sci. *44*, 217–218 (1963).
4. Sims, R. P. A., W. G. McGregor, A. G. Plessers and J. C. Mes, JAOCS *38*, 276–279 (1961).
5. Huber, R. E., and S. Zalik, Can. J. Biochem. Physiol. *41*, 745–754 (1963).
6. Dybing, C. D., and D. C. Zimmerman, Plant Physiol. *41*, 1465–1470 (1966).
7. Dybing, C. D., Crop Sci. *8*, 313–316 (1968).
8. Zeman, I., and V. Kratochvil, Biol. Plant. *9*, 1–14 (1967).
9. Hill, A. B., and P. F. Knowles, Crop Sci. *8*, 275–277 (1968).
10. Hopkins, C. Y., and M. J. Chisholm, Can. J. Biochem. Physiol. *39*, 1481–1487 (1961).
11. Klopfenstein, W. E., and Y. Pomeranz, Lipids *3*, 557–560 (1968).
12. McKillican, M. E., and R. P. A. Sims, JAOCS *40*, 108–113 (1963).
13. McKillican, M. E., Ibid. *43*, 461–465 (1966).
14. Daftary, R. D., and Y. Pomeranz, J. Food Sci. *30*, 577–582 (1965).
15. Evans, J. W., Cereal Chem. *18*, 468–473 (1941).
16. Brimhall, B., and G. F. Sprague, Ibid. *28*, 225–231 (1951).
17. Leng, E. R., Crop Sci. *7*, 333–334 (1967).
18. Bligh, E. G., and W. J. Dyer, Can. J. Biochem. Physiol. *37*, 911–917 (1959).
19. Hirsch, J., and E. H. Ahrens, Jr., J. Biol. Chem. *233*, 311–320 (1958).
20. Mangold, H. K., JAOCS *38*, 708–727 (1961).
21. Horning, E. C., E. H. Ahrens, Jr., S. R. Lipsky, F. H. Mattson, J. F. Mead, D. A. Turner and W. H. Goldwater, J. Lipid Res. *5*, 20–27 (1964).
22. McKillican, M. E., JAOCS *41*, 554–557 (1964).

[Received April 14, 1969]