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FATTY ACID COMPOSITION OF OIL DURING MAIZE KERNEL DEVELOPMENT

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Abstract—Dry wt, total oil and fatty acid composition of the oil was determined during kernel development of three maize inbreds. There was significant variability among these inbreds for duration and rate of dry wt, oil and fatty acid accumulation. Relative quantities of the component fatty acids changed as the kernels developed. Palmitic and linolenic decreased while oleic and stearic increased with maturity. Inbred C103, the higher oil inbred, appeared to accumulate oil over a longer period of time while inbred Hy2, the lower oil inbred, accumulated oil over a shorter period of time. However, the latter had higher daily rates of synthesis for some of the unsaturated fatty acids.

INTRODUCTION

CORN (*Zea mays* L.) oil generally contains palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid. Quackenbush *et al.*[1] surveyed 125 inbred lines and reported that the iodine values, which are dependent upon the ratio of unsaturated acids to saturated acids, ranged from 111 to 151. Studies of the development of corn oil quality by Evans[2] and Brimhall and Sprague[3] showed that iodine value increased with maturity but stabilized long before the kernels reach their full size.

Previous reports[4–7] have established that the fatty acid distributions were primarily determined by the genetic complement of the individual kernels rather than by the physical environment of the parent plants. Curtis *et al.*[8] examined fatty acid distribution in five genotypes at 24 days after pollination and at maturity. Palmitic acid percentage always decreased with maturity, and in three of the five genotypes, oleic acid percentage decreased and linoleic acid percentage increased. Lambert *et al.*[9] sampled the stages of kernel development in two inbred lines and reported that oleic and palmitic acid percentages increased and decreased respectively as the kernels matured and that the percentage composition stabilized by the 25th day after pollination for both inbreds.

The changes in fatty acid composition of the various lipid classes in maturing whole corn kernels has been examined[10] with later emphasis on changes in polar lipids.[11] In the earlier report, the fatty acid composition of various lipid classes extracted from three corn inbreds was examined and the largest increase in fatty acid synthesis occurred between 15

[1] F. W. QUACKENBUSH, J, G. FIRCH, A. M. BRUNSON and L. R. HOUSE, *Cereal Chem.* **40**, 250 (1963).
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[9] R. J. LAMBERT, V. REICH and L. S. SANGRO, *Bol. Inst. Invest. Madr.* **27**, 63 (1967).
[10] EVELYN J. WEBER, *J. Am. Oil Chem. Soc.* **46**, 485 (1969).
[11] EVELYN J. WEBER, *J. Am. Chem. Soc.* **47**, 340 (1970).

and 30 days after pollination.[10] The total amounts of all fatty acids increased in actual weight following pollination, but the pattern of accumulation varied between inbreds. In the later publication[11] it was reported that the quantity of glycolipids and phospholipids increased for a time as the whole kernel matured with a peak occurring between 30 and 45 days after pollination. Changes in fatty acid composition during kernel development were similar for all lipids; palmitic and linolenic acid percentages decreased while oleic acid percentage increased.

In studying fatty acids in the pericarp, endosperm and germ during kernel maturation, Jellum[12] observed significant differences among these fractions. Palmitic percentage of total oil decreased in the pericarp and germ fractions as the kernels matured while oleic and linoleic percentages increased. Linolenic percentages decreased in the germ fraction. The results reported by Jellum[12] generally agree with those of Weber.[10]

The objective of our study was to determine the effects of rate and duration of individual fatty acid synthesis on fatty acid composition of the mature grain of three inbred lines based on actual changes in weight of the oil in whole kernels.

TABLE 1. AVERAGE KERNEL DRY WT AND OIL WEIGHT ACCUMULATION DURING KERNEL DEVELOPMENT OF THREE MAIZE INBREDS

Days after pollination	Av. kernel dry wt (mg)			mg oil per av. kernel		
	C103	B37	Hy2	C103	B37	Hy2
10	11·0 g*	12·2 f	—†	0·12 f	0·10 f	—
14	24·3 g	25·5 ef	19·0 f	0·25 f	0·54 ef	0·27 e
18	71·7 f	55·7 d	54·7 e	2·36 e	1·41 d	0·88 d
22	116·0 e	102·0 c	100·3 d	4·54 d	3·90 c	1·96 c
30	171·3 d	174·5 b	150·0 c	8·10 c	7·75 b	4·68 b
35	204·7 c	208·0 a	187·0 b	10·03 b	9·23 a	6·21 a
40	231·0 b	212·0 a	—	10·73 ab	9·73 a	—
MAT	265·7 a	216·5 a	258·0 a	11·62 a	9·18 a	6·34 a

* Values not followed by the same letter are significantly different at $p = 0{\cdot}05$. Comparisons are within vertical categories.

† Insufficient sample size for 10 and 40 days after pollination.

RESULTS

Kernel Weight

For each inbred line, kernel weight increased from the earliest to the latest sampling date (Table 1). However, by 35 days after pollination more than 96% of the total weight had been amassed for inbred B37. For inbred Hy2, more than 35 days were required to reach a corresponding stage and for inbred C103 more than 40 days were required. The kernel weights of inbreds Hy2 and C103 were greater for these inbreds than for B37. The increase in average kernel dry weight was linear between 18 and 35 DAP. On a milligram per average kernel per day basis, B37, C103 and Hy2 accumulated 9·0, 7·8 and 7·8 mg of dry wt respectively for this 17-day period.

[12] M. D. JELLUM, *J. Am. Oil Chem. Soc.* **47**, 245 (1970).

Oil Content

Based on milligrams per average kernel the oil contents were 6·34, 9·18 and 11·62 in the mature samples for inbreds Hy2, B37, and C103, respectively (Table 1). Similar to kernel weight accumulation, the weight of oil for inbred B37 reached a maximum prior to the final sampling date. Inbreds B37 and Hy2 did not accumulate more oil after the 35-day sampling date while inbred C103 had accumulated only 92·2% of its final oil weight at 40 DAP.

During the period of linear growth between 18 and 35 DAP, oil was accumulated at the rates of 0·31, 0·45 and 0·46 mg oil/av. kernel/day for inbreds Hy2, C103 and B37, respectively. Thus inbred Hy2 synthesized less oil on a daily basis than did the other two inbreds. Inbred C103 synthesized at about the same rate as B37 during period of linear growth but entered the period with higher oil content and continued synthesis over a longer period of time resulting in higher oil content per kernel than the latter (Table 1).

TABLE 2. PALMITIC, STEARIC AND ARACHIDIC ACID ACCUMULATED IN OIL DURING KERNEL DEVELOPMENT OF THREE MAIZE INBREDS

Days after pollination	Fatty acid composition μg per av. kernel								
	Palmitic (16:0)			Stearic (18:0)			Arachidic (20:0)		
	C103	B37	Hy2	C103	B37	Hy2	C103	B37	Hy2
10	23 e*	22 g	—†	2 e	1 f	—	2 d	1 c	—
14	79 e	85 f	54 e	9 de	10 e	4 d	5 d	10 c	4 b
18	340 d	222 e	153 d	40 d	25 d	16 cd	23 cd	21 c	8 b
22	641 c	628 d	406 c	76 c	65 c	42 b	51 bc	61 b	17 b
30	997 b	1215 bc	539 b	157 b	109 b	50 b	99 b	117 a	27 b
40	1365 a	1358 a	704 a	219 a	152 a	73 a	167 a	106 a	25 b
MAT	1314 a	1155 c	593 b	254 a	145 a	85 a	173 a	130 a	66 a

* Values not followed by the same letter are significantly different at $p = 0{\cdot}05$. Comparisons are within vertical categories.

† Insufficient sample size for Hy2 for 10 and 40 days after pollination; 35-day sample substituted in this table for 40 days after pollination.

Palmitic acid (16:0). Palmitic acid, which constituted about 20% of the oil in the 10-DAP samples, ranged from 9·4 to 12·6% of the oil in the mature samples. In absolute terms this represented about 20 μg per kernel at 10 DAP for the two inbreds sampled and 593, 1155 and 1314 μg/per kernel for inbreds Hy2, B37 and C103, respectively, at maturity (Table 2). For each inbred, palmitic acid accumulation continued until 40 DAP. Further kernel development resulted in a reduction of palmitic acid levels for each inbred although this decrease was not statistically significant for inbred C103. The reduction of this fatty acid during the later sampling dates may possibly be due to conversion to other fatty acids or metabolism of this acid by the kernel embryo. Most of the oil is located in the scutellar tissue of the kernel embryo which would make it accessible to metabolic processes occurring in the embryo. The rate of palmitic acid synthesis, as determined by daily accumulation also varied among the inbreds. The rate for inbred C103 was greatest over a longer period of time than that of the other inbreds. The daily accumulation was usually smallest for Hy2.

Stearic acid (18:0). Stearic acid accumulated for longer periods of time and at faster rates for inbreds C103 and B37 than for inbred Hy2 (Table 2). Inbred C103 usually had the greatest daily accumulation. B37 appeared to slow stearic acid accumulation earlier than C103 as evidenced by the slight decrease between the 40 DAP and maturity (Table 2).

Arachidic acid (20:0). Arachidic acid accumulation in inbred Hy2 did not change significantly over the period of linear kernel growth, however, it did increase during final maturation (Table 2). Significant accumulation of this acid also stopped earlier for inbred B37 than for C103.

Oleic acid (18:1). Each inbred exhibited a maximum accumulation of oleic acid prior to maturity although the reduction in the last sample was significant only for B37. Inbred C103 continued oleic synthesis for a longer period than did the other inbreds and at a much faster daily rate (Table 3). Relative amounts of oleic acid in the mature samples ranged from 16·8% for inbred Hy2 to 38·6% for inbred C103 while B37 had 24·0%. In each case this represented an increase in the 10-day oleic acid percentage which varied from 6·9 to 11·8%.

TABLE 3. OLEIC, LINOLEIC AND LINOLENIC ACID ACCUMULATED IN OIL DURING KERNEL DEVELOPMENT OF THREE MAIZE INBREDS

Days after pollination	Fatty acid composition μg per. av. kernel Oleic (18:1)			Linoleic (18:2)			Linolenic (18:3)		
	C103	B37	Hy2	C103	B37	Hy2	C103	B37	Hy2
10	14 e	10 h	—	61 g	51 e	—	13 e	12 c	—
14	227 e	119 g	35 d	257 fg	281 e	152 d	23 e	30 c	19 d
18	794 d	335 e	149 d	1076 e	734 d	501 d	63 de	61 c	47 c
22	1505 c	904 d	688 c	2140 d	2079 c	2079 c	108 cd	119 b	129 b
30	3382 b	1752 c	901 b	3250 c	4298 b	3997 b	141 bc	199 a	145 ab
40	4308 a	2408 a	1173 a	4448 b	5427 a	4054 a	188 ab	219 a	158 a
MAT	4498 a	2198 b	1064 a	5091 a	5269 a	4373 a	221 a	222 a	124 b

* Values not followed by the same letter are significantly different at $p = 0{\cdot}05$. Comparisons are within vertical categories.

† Insufficient sample size for Hy2 for 10 and 40 days after pollination; 35-day sample substituted in this table for 40 days after pollination.

Linoleic acid (18:2). Linoleic acid was the predominant fatty acid in the oil of inbreds C103 and B37 at 10 DAP. As the kernels grew, linoleic acid continued to be synthesized but the amount accumulated relative to the other fatty acids decreased for C103 and increased for B37 and Hy2 (Table 3). Generally, the daily accumulation rate was higher for B37 than for the other two inbreds. There were very large increases in amounts of this acid during the linear growth phase of all inbreds. For inbreds C103 and Hy2 the weight of linoleic acid continued to increase until the final sampling date, therefore, more than 40 days were required to accumulate linoleic acid. For inbred B37, the accumulation of linoleic acid peaked at 40 DAP.

Linolenic acid (18:3). The percentage of linolenic acid in the inbreds at 10 DAP varied from 11·1 to 12·4% and from 1·9 to 2·4% in the mature kernels. Except for inbred Hy2 the percentage of linolenic acid stabilized at 26–30 DAP.

Accumulation of linolenic acid continued until 40 DAP for inbred C103, whereas this acid remained relatively constant following the 30-day sample for inbred B37. Inbred B37 also synthesized this unsaturated fatty acid over a shorter period of time and at a faster rate than C103 or Hy2. Linolenic acid accumulation reached a maximum at 35 DAP which was followed by a substantial decrease for inbred Hy2 (Table 3). As with the other fatty acids, inbred C103 appeared to synthesize linolenic acid for a longer period than either inbred B37 or Hy2, thus accounting for an equal or greater accumulation of this fatty acid in C103. For inbred Hy2, the apparent loss of linolenic acid following the 35-day sampling date may have been due to metabolism or conversion to other fatty acids.

DISCUSSION

Three factors were found to influence the final oil content and fatty acid distribution of the oil in corn. The first was the duration of synthesis for oil and fatty acids. The time from start to finish of active synthesis of oil and fatty acids varied from inbred to inbred. For example, inbred C103 continued linoleic accumulation longer than did the other two inbreds (Table 3).

Second, for inbreds B37 and Hy2, some fatty acids that were accumulated early in the sampling period were apparently metabolized later. Such losses are suggested by the decreases in actual weight of the fatty acids in the late stages of kernel development. The oil is presumably synthesized and stored by the kernels for eventual use as energy during germination, respiration and other processes. Also, it is possible that conversion to other fatty acids might occur and, thus, contribute to selective decreases in some oil components.

Third, and of primary importance, is the variation in rate of synthesis between inbreds for oil or a particular fatty acid. The rates indicate numerous differences between inbreds. The rates of accumulation should be influenced primarily by amounts and types of enzymes catalyzing the various synthetic processes.

If it is desirable to produce corn varieties with high oil percentages that are also highly unsaturated, long-duration oil synthesis such as in inbred C103 should be combined with the highly efficient system for production of unsaturated acids as in inbred Hy2. Apparently rate of oil synthesis and/or duration of oil synthesis can be increased by selection for high oil. Leng[13] reported that oil synthesis continued for nearly 50 days in the selection Illinois High Oil. Unfortunately oil of the Illinois High Oil selection contains only about 48% linoleic acid[8] and, therefore, is not highly valued as a commercial product. It is possible, however, that biosynthesis of more unsaturated oil could be combined with high oil production, especially if the proper germplasm is introduced.[7]

EXPERIMENTAL

Plant material. The inbred corn lines C103, B37 and Hy2 were grown during the summer of 1968 at the University of Kentucky Agricultural Experiment Station farm. These inbreds were selected because of their distinctive fatty acid distributions as determined by preliminary studies. The percent linoleic acid in their oil was approx. 50, 60 and 70, respectively. 50 plants of each inbred were self-pollinated on the same day. Ear samples were taken at 10, 12, 14, 16, 18, 22, 26, 30, 35 and 40 days after pollination and after maturity. The final sampling date was also the same for the three inbreds. Physiological maturity as measured by black layer formation[14] was 46, 48 and 50 days after pollination (DAP) for B37, C103 and Hy2, respectively. At each sampling date four ears were taken from inbred B37 and three from inbreds C103 and Hy2. The kernels from each sample ear were removed with a curved wood gouge and stored at −65° until analyzed for total oil and fatty acid content.

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[14] T. B. DAYNARD and W. G. DUNCAN, *Crop Sci.* **9**, 473 (1969).

Total oil analysis. After the moisture was removed by lyophilization, a sample of 20 whole kernels from each ear was freed of glumes and weighed. 1 g of each dry immature sample was analyzed by NMR for total oil content.[7] 10 single kernels were analyzed for total oil from each mature sample ear. The values for total oil are expressed in terms of mg of oil per average kernel. The daily oil and dry weight accumulation (mg/kernel/day) were calculated for the linear growth phase by substracting the quantities at the beginning from those at the end and dividing by the number of days.

Fatty acid analysis. Samples from both the mature and immature sample ears were analyzed for component fatty acids by GLC. The oil was extracted by homogenizing for 30 sec with 20 ml light petrol., filtering and evaporating to dryness. The fatty acids were then esterified by base-catalyzed interesterification.[7,15] GLC analyses utilized a 3 m × 4 mm i.d. glass column packed with 12% diethylene glycol succinate on 70/80 mesh Anakrom ABS. Operation temperatures for the injection port, column oven, and flame ionization detector were 250, 185 and 250°, respectively. Carrier gas flow rate was maintained at 50 ml/min and peak areas were quantitated by standard triangulation methods. The values for fatty acids are expressed in terms of mg of each per average kernel. The calculations were made by multiplying the relative contribution (percentage) of each fatty acid by the oil content (mg) of the average kernel at each sampling date.

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[15] H. KURTZ, *Fette Seifen* **44**, 144 (1937).