The Lipids of Corn Germ and Endosperm[1]

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ABSTRACT

Mature kernels of an inbred corn were hand dissected into germ and endosperm fractions. Among various solvents tested, boiling, water-saturated *n*-butanol extracted the most lipid from endosperm, and it was used as the extracting solvent for both germ and endosperm. The germ contained 78% of the total lipids and the endosperm 17%. The most striking differences in the fatty acid compositions of the triglycerides and polar lipids were higher levels of stearic and linolenic acids in the endosperm lipids. Although precautions were taken during extraction to inactivate lipases, immediately after harvest the free fatty acid level of the total lipids of the whole kernel was 6.5%. Ninety-five percent of the free fatty acids was in the endosperm fraction where the free fatty acids made up 36.5% of the total lipids. In germ, free fatty acids represented only 0.6% of the total lipids. The individual phospholipid and glycolipid classes of the endosperm and germ lipids were similar except for high levels of lyso compounds in the endosperm lipids. The higher levels of linolenic acid, free fatty acids and lyso lipids in endosperm may affect the keeping quality of the corn grain and of fractions milled from the endosperm.

INTRODUCTION

Almost all of the previous studies of corn lipids have been essentially studies of corn germ lipids, because the major portion of corn lipids is found in the germ. In 11 varieties of corn, Earle et al. (1) showed that 81-87% of the corn oil was extracted from the germ fractions. The oil contents were high (31-39%) in the germ fractions and low (0.7-1.1%) in the endosperm fractions. Thus, even in extractions of whole kernels, the characteristics of the endosperm lipids would be obscured by those of the germ lipids.

In commercial milling, corn oil from the germ fraction is the important product. Little consideration has been given to the lipids in the endosperm, although these lipids may affect the properties and keeping quality of the various milling fractions derived from the endosperm. For example, in comparisons of defatted and nondefatted cereal starches (2), differences were noted in water-binding capacities, swelling powers, solubilities, gelatinization temperature ranges, and amylograph viscosities. Lipids also are known to have essential roles in baking flours (3).

Jellum et al. (4-6) and Jahn-Deesbach and coworkers (7) showed that the fatty acid compositions of the lipids extracted from germ and endosperm were different, but they did not investigate the types or quantities of lipids that were present. Morrison (8) has calculated the amounts of the nonpolar lipids, glycolipids and phospholipids in corn germ lipids and endosperm nonstarch and starch lipids from their fatty acid methyl esters. In this study, determinations were made of the quantities of triglycerides, free fatty acids and the individual lipid classes present in the phospholipids and glycolipids of germ and endosperm.

Quantitative extraction of endosperm lipid is difficult, because the lipids are complexed with proteins and starch. Jellum (9) noted that oils obtained from corn endosperm by different extraction procedures were significantly different in fatty acid composition while all the oils extracted from the germ were similar. Various solvents have been compared for the extraction of lipids from wheat flour or wheat starch (10,11), and water-saturated *n*-butanol was selected as the preferred solvent. Several solvent systems were tested in this study to determine which system gave optimum extraction of lipids from corn endosperm.

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MATERIALS AND METHODS

Materials

The corn inbred, H51, was grown on the Agronomy Department farm at the University of Illinois, Urbana, IL. Mature kernels were harvested at 60-62 days after pollination. Each kernel was hand dissected. First the tip cap was removed below the black layer. Next the kernel was placed in boiling water for 2 min to inactivate lipolytic enzymes and to facilitate the removal of the pericarp. Under a 1¾x magnifier, the germ was carefully dissected from the endosperm with a scalpel. The endosperm was broken up coarsely in a hand mill. Each germ and endosperm was placed in the extraction solvents as quickly as possible.

Extraction Procedures

Three extraction procedures were tested as to their efficiency in extracting total lipids and free fatty acids from endosperm tissue: 1) extraction with chloroform/methanol/water according to the Bligh and Dyer procedure (12) included reextraction of the tissue with chloroform; 2) extraction with boiling isopropanol followed by the Bligh and Dyer extraction was investigated, with each endosperm extracted for 1 min in 5 ml of boiling isopropanol containing .002% butylated hydroxytoluene (BHT); 3) extraction with boiling, water-saturated *n*-butanol was tested. Each endosperm was extracted for 3 min in 2.5 ml of boiling, water-saturated butanol containing .002% BHT. The extracts and endosperm tissues were transferred to a homogenizer flask and left at room temperature until 50 endosperms had been collected. The endosperms were homogenized with a Virtis 45 tissue grinder. The endosperm tissue was filtered from the extract on a Buchner funnel. The extraction of the endosperm tissue for 3 min with boiling, water-saturated butanol, homogenization and filtration was repeated to give a total of three extractions. This procedure was also adopted for extraction of the germ tissue with boiling, water-saturated butanol, but the volume of butanol was reduced to 1 ml for each germ.

The lipid extracts were evaporated to dryness in a rotary evaporator. The residues were taken up in 0.5-1 ml of chloroform/methanol (19:1, v/v) plus water (10 drops) and reduced once more to dryness to break lipoprotein associations. The residues were taken up again in a small volume of chloroform/methanol/water (190:10:1) for partition chromatography.

Column Chromatography

The lipids were purified by chromatography on Sephadex G-25 columns (13,14). The nonlipid contaminants of the Sephadex column fractions were assessed by micro-Kjeldahl nitrogen determinations (15) and sugar analyses. To prevent interference by sterols, the samples were hydrolyzed in 2N sulfuric acid at 100 C (16) and extracted with petroleum ether (b.p. 60-68 C) (17) before sugar analysis

TABLE I

Comparison of Various Solvents in the Lipid Extraction of Corn Endosperm

Boiling water before dissection	Extraction procedure	FAME[a]	Free fatty acid
min		% of dry wt	% of FAME
2	Bligh-Dyer[b]	0.84 ± 0.02[c]	36.8 ± 0.7
2	Boiling isopropanol, Bligh-Dyer	0.88 ± 0.03	33.0 ± 0.8
10	Boiling isopropanol, Bligh-Dyer	0.85 ± 0.01	31.6 ± 0.3
2	Boiling water-saturated *n*-butanol	1.04 ± 0.01	37.4 ± 1.4

[a]FAME = Fatty acid methyl ester weight.

[b]See text and reference 12.

[c]Mean ± standard error of mean.

TABLE II

Lipids from Whole Corn Kernel, Germ and Endosperm of Inbred H51

	Whole kernel	Germ	Endosperm
Dry weight, mg	233	25	192
% of kernel	—	10.9	82.6
Total lipid extract, %			
Wt[a]/dry wt	n.d.[b]	45.3	6.8
FAME[c] wt/dry wt	4.9	35.5	1.0
% of total lipid FAME			
Triglycerides	90.0	93.3	52.4
Free fatty acids	6.5	0.6	36.5
Polar lipids	3.5	2.6	10.9

[a]Wt - Gravimetric weight that includes extraneous material.

[b]n.d. - not determined.

[c]FAME - fatty acid methyl esters.

by the phenol-sulfuric acid procedure (18).

The total lipids were separated on silicic acid columns (19). Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol.

Thin Layer Chromatography and Determination of Individual Lipids

The lipids were identified by comparing their thin layer chromatographic behavior with authentic samples, published data (10,20) and reactions with various spray reagents (21). Phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), lysophosphatidylcholine (LPC), steryl glucoside ester (SGE), monogalactosyldiglyceride (MGDG), cerebrosides (CER), and steryl glucoside (SG) were obtained from Supelco, Inc., Bellefonte, PA; phosphatidylserine (PS), phosphatidylinositol (PI), lysophosphatidylethanolamine (LPE) from Applied Science Laboratories, Inc., State College, PA; free fatty acid (FFA) and phosphatidic acid (PA) from Sigma Chemical Co., St. Louis, MO; phosphatidylglycerol (PG) from Serdary Research Laboratories, Inc., London, Ontario, Canada. The standards for triglyceride (TG), phosphatidylcholine (PC) and digalactosyldiglyceride (DGDG) were isolated from corn lipids in our laboratory. N-acyl phosphatidylethanolamine (N-acyl PE) was synthesized from corn PE (22).

The phospholipids were separated by two dimensional thin layer chromatography (TLC) on silica gel H 0.3 mm thick. The solvent systems were chloroform/methanol/[ammonia (29.3% w/v)] (65:35:5) and chloroform/acetone/methanol/acetic acid/water (10:4:2:2:2). Duplicate plates were run on each of two separate extractions. The phospholipid spots were visualized with iodine and analyzed for phosphorus in the presence of silica gel (23).

The glycolipids were chromatographed in one dimension by chloroform/methanol/ammonia (70:20:1). The sugar content of each glycolipid band was determined by the orcinol procedure of Svennerholm (24) modified for direct digestion of the glycolipids in the presence of silica gel (21).

Gas Chromatography

The methyl esters of the fatty acids were prepared by treatment of the lipids with boron trifluoride/methanol according to the procedure suggested for each type of lipid by Morrison and Smith (25). The methyl esters were analyzed by a Hewlett-Packard Model 5830A gas chromatograph (Hewlett-Packard, Avondale, PA), fitted with a flame ionization detector and 1.8 m x 2 mm ID column packed with 15% DEGS on 80/100 mesh Chromosorb W AW (Supelco, Inc., Bellefonte, PA). The column temperature was 172 C with a helium carrier gas flow rate of 30 ml/min. The internal standard was methyl heptadecanoate.

RESULTS

The first concern was facile separation of the kernel parts without enzymatic changes of the lipids. Many workers who have studied seed parts soak the seed for 4-5 hours, overnight or for days, which is essentially a germination step. For corn, placing the kernel in boiling water for only 2 min allowed a clean separation of the pericarp and germ from the endosperm. If the kernel was left in boiling water for 10 min, separation of the parts was difficult. The endosperm was gummy and could not be broken up as well for extraction, and this resulted in lower yields of lipids and of free fatty acids (Table I). Short term boiling also appears to be the most effective way to inactivate lipases without deleterious effects on the lipids (10,26,27).

The efficiencies of various solvents in extraction of lipids from corn endosperm were assessed by comparing the yields of fatty acid methyl esters (FAME) and of free fatty acids (FFA) (Table I). The extraction solvents used were: chloroform/methanol/water in final proportions of 2:2:1.8 (v/v/v), the Bligh and Dyer procedure (12); boiling isopropanol for 1 min followed by a Bligh and Dyer extrac-

TABLE III

Sephadex Column Fractions from Boiling Water-Saturated Butanol Extracts

Sephadex fraction	Whole kernel %		Germ %		Endosperm %	
	Wt[a]	FAME[b]	Wt	FAME	Wt	FAME
I	52.0	98.3	78.8	99.3	18.5	98.8
II-A	18.1	1.2	2.0	0.7	55.9	1.2
-B	16.8	0.5	0.2	---	15.7	---
III	13.1	---	19.0	---	9.9	---
Recovery of initial wts	n.d.[c]	98.8	99.3	100.8	81.0	89.7

[a]Wt = gravimetric weight.

[b]FAME = fatty acid methyl ester weight.

[c]n.d. = not determined.

TABLE IV

Nitrogen and Sugar Content of Sephadex Fractions from Corn Germ and Endosperm

Sephadex fraction	Germ			Endosperm		
	N %	Protein[a] %	Sugar %	N %	Protein %	Sugar %
I	n.d.[b]	---	0.3	n.d.	--	0.5
II-A	9.8	55.9	2.6	15.8	90.1	0.4
-B	4.8	27.4	14.4	15.9	90.6	5.2
III	1.8	10.3	87.4	3.2	18.2	51.8

[a]Protein % = N % x 5.7.

[b]n.d. = not determined.

tion; boiling, water-saturated *n*-butanol for 3 min. The lowest yield of FAME (0.84%) was obtained with the Bligh and Dyer procedure. The higher temperature extractions gave better yields. Boiling *n*-butanol gave the highest values both for FAME (1.04%) and for FFA (37.4%). Therefore, boiling, water-saturated *n*-butanol was chosen as the solvent for extraction of the lipids from the whole kernel, germ and endosperm (Table II).

This corn inbred had 4.9% oil, as measured by FAME, in the whole kernel. The germ made up only 10.9% of the dry weight of the kernel, but a large percentage, 35.5%, of the germ was lipid. The germ contained 78% of the total lipid. By far the major portion of the corn kernel was endosperm, 82.6%, but the endosperm was low in lipid, 1%, and even with its large size contained only 17% of the total lipid of the whole kernel. The pericarp made up 6.1% of the whole kernel and the tip cap, 0.7%. Both were very low in lipid, <0.4%.

Not only did boiling, water-saturated *n*-butanol extract the most lipid, but it also extracted much extraneous material. The extent of this problem can be seen in Table II by comparing the percentages of total lipid as wt/dry wt to FAME/dry wt. For germ, the gravimetric weight of the total lipid was 128% of the FAME weight. The problem was much greater in endosperm where the gravimetric weight was 680% of the FAME weight.

As measured by FAME, triglyceride was the major lipid class (Table II), particularly in germ where triglyceride accounted for 93.3% of the total lipids. In endosperm, 52.4% was triglyceride. The amount of FFA in the endosperm lipid was surprising; 36.5% of the total lipid in endosperm was FFA. In germ, only 0.6% of total lipid was FFA. Approximately 95% of the total FFA was found in the endosperm. The precautions taken to avoid lipase activity during dissection appeared to be effective, because the recovery of endosperm plus germ FFA was 101% of the FFA of the whole kernel. For the polar lipids, the FAME percentage of the total lipid was higher in endosperm (10.9%) than in germ (2.6%). However, when the yield of total lipid from each tissue was considered, approximately half of the polar lipid FAME was found in the germ and half in the endosperm.

Sephadex G-25 columns were used to remove the nonlipid contaminants. The distribution of weight and of FAME in the Sephadex column fractions (Table III) gave some clues as to the recovery of lipid and the nature of the contaminants in the lipid extracts. FAME values greater than 98% indicated that the recovery of lipid in Fraction I was nearly complete for whole kernel, germ and endosperm. The gravimetric weight distribution among the Sephadex column fractions for endosperm showed only 18.5% of the weight in fraction I and the major portion, 55.9%, in fraction II-A. Nitrogen analysis indicated 90% protein and amino acids in endosperm fractions II-A and B (Table IV). The major storage protein of the endosperm, zein, is alcohol soluble and could be extracted by butanol. Carbohydrates were the major components in fraction III of the Sephadex columns.

Of the initial endosperm FAME placed on the Sephadex columns, 89.7% was recovered (Table III). Lipids may have been lost by being occluded in nonlipid material which remained insoluble on the top of the columns. Only 81% of the original weight was recovered from the endosperm columns.

The lipid fraction, fraction I of the Sephadex columns, was subdivided into three fractions on silicic acid columns (Table V). The neutral lipids made up a larger proportion of the total lipids in the germ (92.4%) than in the endosperm (86.9%). For glycolipids, the weight percentage in the endosperm was 2.7 times that of the germ and for phospholipids, 1.3 times greater in endosperm.

The fatty acid compositions of the lipids separated by the silicic acid columns are shown in Table VI. The endosperm lipids tended to have more saturated fatty acids, particularly stearic acid, than germ lipids, but the most distinctive feature of the endosperm lipids was the higher percentage of linolenic acid. The presence of this triunsaturated fatty acid may affect the keeping quality of commercial products prepared from the endosperm. The glycolipids and phospholipids had more saturated fatty

TABLE V

Silicic Acid Column Chromatography of Corn Germ and Endosperm Lipids

Eluant	Lipids	Germ	Endosperm
		% of wt of total lipids	
Chloroform	Neutral	92.4	86.9
Acetone	Glycolipid	2.0	5.4
Methanol	Phospholipid	5.6	7.6
Recovery of initial wts		97.9	101.6

TABLE VI

Fatty Acid Composition of Lipids Separated by Silicic Acid Columns

	Fatty acid composition, Mole %				
	16:0	18:0	18:1	18:2	18:3
Germ					
Neutral lipids	16.6	1.3	31.8	49.5	0.8
Glycolipids	24.9	1.7	28.3	42.8	2.3
Phospholipids	24.9	1.3	31.1	42.0	0.7
Endosperm					
Neutral lipids	20.8	2.4	23.6	49.4	3.8
Glycolipids	28.1	2.8	20.0	44.6	4.5
Phospholipids	21.3	2.4	35.1	39.6	1.6

acids than the neutral lipids in both endosperm and germ, but the highest percentage of linolenic acid was in the glycolipids.

The most striking difference between endosperm and germ phospholipids was the high level of lysophospholipids in the endosperm (Table VII). Lysophosphatidylcholine and lysophosphatidylethanolamine accounted for 38.5% of the phospholipid in endosperm. Phosphatidylcholine was the major phospholipid in both germ (54.5%) and endosperm (44.6%). The percentages of phosphatidylinositol and phosphatidylethanolamine were higher in germ than in endosperm.

The glycolipid fractions from the silicic acid columns were analyzed for phosphorus, because N-acyl phospholipids had been reported previously in this fraction in wheat (10). Only 2.5% of the total lipid phosphorus was present in the germ glycolipid fraction and 2.9% in the endosperm fraction. Very little phospholipid was eluted into the glycolipid fractions in corn.

Among the glycolipids, the levels of both monogalactosyldiglyceride and digalactosyldiglyceride were higher in germ than in endosperm (Table VIII). A lyso compound, lysomonogalactosyldiglyceride, was again found in endosperm, but none was detected in the germ. Lysodigalactosyldiglyceride may also be present at the origin or just above the origin in the sulfolipid band, but it has not been identified as yet. Other glycolipids that may occur in these bands are the polyglycolipids such as the tri- and possibly tetraglycosyldiglycerides that have been isolated from rice (28). The sugar-containing band at the front may be an acylated monogalactosyldiglyceride. This lipid class has been identified in wheat flours (11).

DISCUSSION

In commercial milling, the quantity and quality of the oil in corn germ has received the major attention, but the types and fatty acid compositions of the lipids from other parts of the kernel should also be considered. These lipids may significantly influence the properties of the various milling fractions. Keeping quality, for example, may be greatly affected.

Both qualitative and quantitative differences have been found between the lipids of germ and endosperm in this corn inbred. The most interesting aspect of the endosperm lipids is the role of FFA and lyso lipids. In the endosperm, both lyso lipids and FFA were found, but FFA made up the major monoacyl lipid class. Morrison (29) analyzed the lipids of commercial corn starch and found that 62% of the lipid was FFA and 18% lysophosphatidylcholine. By contrast, in wheat, barley, rye and oat starches, the lipids were mainly lysophospholipids (52-62% lysophosphatidylcholine) with only 2-12% FFA (30).

A general correlation has been noted between amylose content and the monoacyl lipids of corn starch. More lipid was found in high amylose starches than in normal starches and very little in waxy (high amylopectin) starches (8,31). Some evidence exists which suggests that the lipid may exist as an inclusion complex inside the amylose helix (30,31). It has been proposed that the monoacyl lipids act as initiators or templates for starch granules (29,32). Vieweg and de

TABLE VII

Phospholipids of Corn Germ and Endosperm

Spot No.	Phospholipid	Germ	Endosperm
		% of total lipid P	
13	N-acyl phosphatidylethanolamine	1.1 ± 0.1[a]	1.2 ± 0.2
12	N-acyl lysophosphatidylethanolamine[b]	1.8 ± 0.2	1.1 ± 0.1
11	Diphosphatidylglycerol	2.1 ± 0.5	0.9 ± 0.1
10	Phosphatidylethanolamine	11.7 ± 0.3	6.1 ± 0.4
9	Phosphatidylglycerol	4.3 ± 0.7	1.9 ± 0.1
8	Phosphatidic acid	1.2 ± 0.1	0.7 ± 0.1
7	Phosphatidylcholine	54.5 ± 2.6	44.6 ± 1.0
6	Lysophosphatidylethanolamine	0.4 ± 0.1	3.4 ± 0.2
5	Phosphatidylserine	0.9 ± 0.1	ud.[c]
4	Phosphatidylinositol	18.7 ± 1.3	3.6 ± 0.2
3	Unknown	1.4 ± 0.2	1.4 ± 0.2
2	Lysophosphatidylcholine	0.8 ± 0.1	35.1 ± 0.7
1	Unknown	1.1 ± 0.1	

[a]Mean ± standard error on mean.

[b]Tentative identification.

[c]ud. = undetected.

TABLE VIII

Glycolipids of Corn Germ and Endosperm

Glycolipid	Germ	Endosperm
	% of total sugar	
(Front)	0.3 ± 0.2[a]	6.2 ± 0.1
Sterylglycoside ester	1.6 ± 1.1	4.1 ± 0.8
Monogalactosyldiglyceride	9.3 ± 1.2	8.1 ± 0.5
Cerebroside + sterylglycoside	32.8 ± 0.1	13.1 ± 0.6
Cerebroside (OH-fatty acids)	22.0 ± 0.1	8.6 ± 1.0
Lysomonogalactosyldiglyceride	ud.[b]	12.5 ± 0.9
Digalactosyldiglyceride	21.0 ± 1.7	15.5 ± 0.7
Sulfolipid (+ ?)	7.0 ± 0.8	19.7 ± 1.3
(Origin)	5.9 ± 0.6	12.3 ± 1.2

[a]Mean ± standard error of the mean.

[b]ud. = undetected.

Fekete (33) found that the "branching enzyme" of corn endosperm did not produce amylopectin from amylose that was complexed with phospholipids. They suggested that some of the newly synthesized amylose chains were protected by formation of a complex with lipids. This protected amylose could not undergo branching or breakdown, but it could be elongated. Amylopectin would only be formed from the chains that were not complexed. These proposals could explain the simultaneous deposition of amylose and amylopectin that occurs in starch granules and the high lipid content of high amylose starch.

Monoacyl lipids may also be important constituents of the protein bodies that are found in endosperms of cereal grains. Hirayama and Matsuda (34) obtained high levels of lysophospholipids from protein bodies of rice endosperm.

Various endosperm mutants, such as opaque-2, floury-2, waxy, high amylose, sugary, shrunken, and brittle (35,36), have been shown to affect the oil content and fatty acid composition of whole kernel corn. The effects of these mutants on the endosperm lipids alone is difficult to assess, because the endosperms were not separated, and the lipid extractions were not done with *n*-butanol.

The lipid compositions of endosperm and germ can be modified by corn breeding. It is important to remember that the inheritance of these morphological parts is different. The hereditary makeup of the endosperm is triploid, two-thirds maternal and one-third paternal, but the germ is diploid, equal inheritance from both parents. In breeding corn, the selection of parents and the direction of the cross will determine the genetic composition of each part. We need to know more about the physical and physiological properties of the lipids in endosperm and germ before we can intelligently select parents for better corn.

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