# The Distribution of Lipids in the Germ, Endosperm, Pericarp and Tip Cap of Amylomaize, LG-11 Hybrid Maize and Waxy Maize

**SIEW LENG TAN** and **WILLIAM R. MORRISON**, Department of Food Science and Nutrition, University of Strathclyde, 131 Albion Street, Glasgow G1 1SD, Scotland, UK

# ABSTRACT

The quantitative distribution of 23 acyl lipid classes and unsaponifiable matter in kernels of amylomaize, LG-11 hybrid maize and waxy maize is described. LG-11 and waxy maize were normal (oil content) varieties, containing 4.9% and 5.1% lipid, respectively, while amylomaize (9.3% lipid) was a high oil variety. The distribution of kernel lipids was 76-83% in germ, 1-2% in pericarp, 1% in tip cap, 1-11% in starch, and 13-15% in aleurone plus the nonstarch fraction of the starchy endosperm. Germ contained 39-47% lipid, which was mostly triglyceride (TG), with some steryl esters (SE) and diglycerides (DG), and small amounts of glycolipids (GL) and phospholipids (PL). Aleurone lipids appeared to be TG with some free fatty acids (FFA) and SE. The other nonstarch lipids in starchy endosperm were FFA with very small amounts of SE, DG, GL and PL. The starches had a little surface lipid (FFA) and true (internal) starch lipid (FFA, lyso-PL) in quantities roughly related to amylose content (amylomaize = ca. 73% amylose, 1.0% lipid; LG-11 = 23% amylose, 0.7% lipid; waxy maize = < 5% amylose, 0.2% lipid). Pericarp lipids (0.8-2.5%) were mainly unsaponifiable matter, the acyl lipids being TG, SE, DG and FFA. Tip cap lipids (2.5-2.9%) had more TG, GL and PL

than pericarp lipids, but were otherwise similar. Pericarp lipids and endosperm nonstarch lipids appeared to have suffered extensive degradation at some time during kernel development or after harvesting, while lipids in starch, germ and tip cap were evidently unaffected. FFA and lyso-PL are regarded as normal components of maize starch (rather than degradation products) and may occur as amylose inclusion complexes.

# INTRODUCTION

A logical starting point for a systematic study of cereal lipids would be the quantitative distribution of all lipid classes in the principal parts of the caryopsis. We are aware of no such data in the literature for any cereal, although there is information on the distribution of total lipids in all cereals, and on sterols, tocopherols, carotenoids and some glycerides in maize, wheat and rice (1).

In view of the importance of maize lipids, and the fact that the maize kernel is large and therefore comparatively easy to dissect, we decided to study the distribution of lipids in maize. We used commercial samples of amylomaize, LG-11 hybrid maize and waxy maize, and determined the distributions of 23 acyl lipid classes in the pericarp, tip cap, germ, endosperm and starch. Our results, reported in this paper, complement previous analyses of the

	Percentage of	Total lipid (% o	f dry wt.)		Percen	tage fatty	acid com	position <sup>b</sup>	
	kernel dry wt.	Hydrolysis <sup>a</sup>	Solventb	16:0	18:0	18:1	18:2	18:3	Others
Amylomaize									
Pericarp	7.8	0.19	0.24	21.8	5.8	23.9	39.5	6.6	2.5
Endosperm, total	74.7	2.42		25.1	3.6	25.1	43.7	2.6	
Endosperm, NSL + aleurone <sup>C</sup>			1.77	18.3	1.9	27.5	49.6	2.7	
Endosperm, NSL – aleurone <sup>C</sup>			0.69	22.4	3.6	21.3	49.4	3.3	
Endosperm SL <sup>c</sup>			0.65	38.5	4.3	20.0	36.0	1.2	
Starch		0.86	0.82	36.2	4.7	20.2	37.8	1.2	
Germ	15.0	37.23	33.74	12.0	2.0	29.3	55.0	1.7	
Tip cap	2.5	1.75	1.76	16.9	4.0	32.9	40.1	6.1	
LG-11									
Pericarp	4.2	0.24	0.29	24.7	5.2	21.1	45.8	3.1	
Endosperm, total	86.0	1.02		24.7	1.9	15.6	53.5	4.3	
Endosperm, NSL + aleurone <sup>C</sup>			0.57	16.6	2.1	19.0	57.8	4.5	
Endosperm, NSL – aleurone <sup>c</sup>			0.29	24.0	1.7	11.6	58.6	4.1	
Endosperm, SL <sup>C</sup>			0.45	31.4	1.1	11.1	53.8	2.7	
Starch		0.56	0.51	36.8	1.8	10.1	47.9	3.5	
Germ	8.4	40.60	38.71	11.0	1.4	24.1	62.8	0.7	
Tip cap	1.4	2.03	1.62	22.1	2.6	20.0	49.2	6.0	
Waxy maize									
Pericarp	6.6	0.22	0.20	19.4	4.5	21.4	40.3	3.0	11.4
Endosperm, total	81.0	0.80		27.5	2.0	15.4	49.9	5.3	
Endosperm, NSL + aleurone <sup>c</sup>			0.73	24.6	1.8	14.0	53.4	6.2	
Endosperm, NSL - aleurone <sup>c</sup>			0.45	33.3	2.2	10.0	50.6	4.0	
Endosperm, SL <sup>c</sup>			0.12	29.5	4.1	23.0	40.2	3.3	
Starch		0.14	0.14	35.5	4.4	23.2	35.5	1.5	
Germ	10.8	35,66	35.57	10.6	1.5	20.3	66.3	1.3	
Tip cap	1.6	1.96	1.71	17.4	2.8	27.9	49.4	2.6	

TABLE I

Total Lipid Content, as Fatty Acid Methyl Esters, of	f Dissected Maize Kernels
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<sup>a</sup>By acid hydrolysis, extraction and methanolysis.

<sup>b</sup>By direct solvent extraction and methanolysis – except total endosperm fatty acids which were by hydrolysis and methanolysis.

<sup>c</sup>NSL = nonstarch lipids, SL = starch lipids.

total lipids (2-6), total fatty acids (3,5,7-10), carotenoids (11) and tocopherols (12) in dissected fractions of maize kernels.

# EXPERIMENTAL PROCEDURES

#### Materials

LG-11 hybrid maize was provided by Dr. R. Drapron, Station de Biochimie et Physico-Chemie des Céréales, I.N.R.A. au C.E.R.D.I.A., Massy, France. A commercial sample of waxy maize was obtained from Dr. D. Pedrina, Fabbriche Riunite Amido Glucosio Destrina, S.P.A., Milan, Italy, and amylomaize from Dr. G.W. Deardorff, American Maize Products Co., Urbana, IL. Reference lipids were prepared from wheat flour (13) or were purchased from Sigma London Chemical Co., Ltd., (Poole, England).

Maize kernels were steeped for 3 hr in 0.1% sodium metabisulfite solution at 50 C before dissection. Endosperm, pericarp and tip cap fractions were air-dried and milled to pass a 100 mesh sieve. Endosperm minus aleurone was prepared by cutting off the pericarp, germ, and the aleurone layer together with some adjacent starchy endosperm from dry kernels. Germ tissue was finely sliced before analyses, with precautions to recover lipid from the scalpel blade.

Starch was prepared from milled endosperm which had been soaked in 0.1% sodium metabisulfite at 50 C for 16 hr. Crude starch was separated by centrifuging at 108,000 x g for 1 hr, and LG-11 starch, essentially free from protein, was obtained by repeated washing and centrifuging. Amylomaize and waxy maize starches required additional steeping at 25 C for 24 hr in Pronase dissolved in 0.2 M phosphate buffer at pH 7.4. The starches were washed in distilled water, air-dried, and ground to pass a 120 mesh sieve.

## Methods

Moisture was determined by drying to constant weight in a vacuum oven at 70 C (minimum 24 hr). All results are expressed on a dry weight basis.

Total acyl lipid was determined as fatty acid methyl esters (FAME) by gas liquid chromatography (GLC) after acid hydrolysis and methanolysis of an aliquot of hydrolyzate lipid, using heptadecanoic acid as internal standard (14). The optimum hydrolysis times were 45 min for endosperm and starch, 360 min for germ, and 90 min for bran and tip cap.

Acyl lipids in aliquots of solvent extracts and on thin layer chromatography (TLC) plates were quantified as FAME, and, in the case of phospholipids, by phorphorus distribution (14).

Unsaponifiable lipids were determined by saponifying 100  $\mu$ l aliquots of lipid extracts with 2 ml of 1N KOMe in MeOH, in tubes sealed under nitrogen and heated at 100 C for 3 hr. After cooling, water (2 ml), diethyl ether (2 ml) and light petroleum (0.6 ml) were added, and the tubes were then well shaken and centrifuged. The upper phase was evaporated dry under nitrogen and redissolved in 1.0 ml chloroform. Aliquots (200 or 300  $\mu$ l) were then evaporated in tarred aluminum foil dishes (ca. 20 mg) and weighed on a Cahn electrobalance.

Optimum conditions for the solvent extraction of lipids were different for each tissue. Endosperm nonstarch lipids were obtained by extracting five times with 10 vol watersaturated n-butanol (WSB) at room temperature, with a total extraction time of 30 min (14). Lipids in purified starch, or the residual starch lipids after extraction of nonstarch lipids, were extracted five times with 10 vol WSB at 90-95 C, with a total extraction time of 6 hr. Bran and tip cap lipids were similarly extracted with hot WSB for 3 hr. Germ was sliced with a scalpel under solvent, and extracted ten times with 5 vol chloroform/methanol (1:1), and the residue was finally extracted with WSB at 90 C for 3 hr. Solvent extracts were concentrated by rotary vacuum evaporation, and the lipids purified by Bligh and Dyer partitioning (15). Lipids were analyzed by silicic acid column chromatography, TLC, and GLC using methods described in previous papers (13,14,16). The data in Tables I-III are accurate to better than  $\pm$  3%, and in most cases to better than  $\pm$  1%.

# RESULTS

#### **Total Lipids**

The weights of the dissected kernel fractions (Table I) show that LG-11 and waxy maize were quite typical, but amylomaize had more pericarp and germ (and less endosperm) than is usual (1,6).

The hydrolyzate lipid and extractable lipid contents (as FAME) of the pericarp and tip cap samples (Table I) were much lower than published lipid contents, but when converted into total lipids, including unsaponifiable matter, the results (Table II) fell within the normal ranges (1,6). Blessin (2) reported 9.5% lipid in the tip cap of five yellow corn hybrids, but the tip cap was known to be contaminated with germ.

More lipid was found in the germ of all three types of maize (Tables I, II) than is usually reported (1,6). This is attributed to the greater efficiency of the anlaytical procedures used in the present study rather than to abnormally high lipid contents.

Endosperm lipids are located in three distinct regions – the aleurone layer, the nonstarch fraction of the starchy endosperm, and inside the starch granules. Hydrolyzate lipid values (Table I) are a measure of the total endosperm lipids. Starch lipid values can only be determined from washed starch, or by first removing the aleurone and other nonstarch lipids from finely milled endosperm samples and then extracting the starch lipids with hot WSB, as is done with wheat flour (14).

Since it was not possible to remove the aleurone layer without the risk of causing unacceptable damage to lipids (e.g., by alkali dehulling), aleurone lipid in amylomaize was determined by difference from complete endosperm and from endosperm minus aleurone and some adjacent starchy endosperm (Table I). This approach was less successful with LG-11 and waxy maize, which contained ca. 280 mg aleurone lipid (as FAME) per 100 g endosperm, compared with ca. 1000 mg in amylomaize. Scanning electron micrographs showed that the aleurone cells in LG-11 and waxy maize were isodiametric, while those in amylomaize were radially elongated and approximately twice as large. Thus, it would be reasonable to attribute the greater aleurone lipid content of amylomaize to its larger proportion of aleurone cells, without necessarily invoking a much higher percentage of lipid within aleurone cells.

Amylomaize also had more lipid than LG-11 or waxy maize in the nonstarch and starch lipid fractions of its starchy endosperm. Starch lipid content decreased with amylose content (ca. 70, 23 and  $\leq 5\%$  amylose, respectively), but was obviously not directly related. This agrees with earlier results of Acker and Schmitz, although their results were generally higher (17).

The total endosperm lipid contents (Table II) were significantly higher than previously reported (2-6). This was probably due to the improved analytical techniques used in the present study, and to the inclusion of starch lipids which were not quantified in previous studies.

The mean dry weights of the kernels were: amylomaize = 249 mg, LG-11 maize = 250 mg, and waxy maize = 284 mg, and the total lipid contents, calculated from Table III, were respectively 9.33, 4.87 and 5.17%. Amylomaize was

LABLE II

thus a high oil variety on account of its greater germ weight and higher lipid content in pericarp and endosperm, while LG-11 and waxy maize had normal lipid contents.

# Germ Lipids

Apart from differences in quantities of unsaponifiable matter, the germ lipids were very similar in all three types of maize (Table II). The principal lipids were triglycerides (70-85% of total), 1,2-diglycerides and steryl esters. The unsaponifiables, which included a large proportion of free sterols, were not analyzed in detail.

The high proportions of phosphatidyl choline and phosphatidyl inositol seem to be characteristic of germ lipids in maize (Table II) and other cereals (18-21), but the amount of phosphatidic acid in amylomaize germ seems unusually high.

# **Endosperm Lipids**

Information on the aleurone lipids is imprecise because they could only be determined by difference, and the endosperm-minus-aleurone material did not represent the whole of the starchy endosperm. Best results were obtained for amylomaize, but the same principal features can be seen in LG-11 and waxy maize (Table II).

Aleurone contained most of the endosperm triglyceride, a substantial part of the steryl ester, diglyceride, free fatty acid (amylomaize only), monoglyceride (amylomaize and waxy maize), phosphatidyl choline and phosphatidyl inositol. There appeared to be little or no glycolipids in the aleurone.

The aleurone lipids resembled germ lipids, as might be expected, because they have similar functions during germination (1). In wheat the aleurone lipids are almost the same as the germ lipids (22) and both have more phospholipids than the corresponding tissues in maize.

The nonstarch lipids (excluding aleurone) were mostly free fatty acids with only small amounts of other nonpolar lipids, glycolipids and phospholipids. This is very unusual, and immediately suggests extensive lipolysis of the diacyl glycerides and triglycerides usually found in the nonstarch lipids of wheat and other cereal endosperm flours (1). Steeping the kernels for dissection did not cause lipolysis, because the same results were obtained from dry dissected kernels. Thus, lipolysis must have occurred during development and drying out of the kernels, or high levels of free fatty acids are a normal feature of the nonstarch lipids in maize endosperm.

Lysophospholipids are the major lipids in wheat and other cereal starches (1,17). They resist extraction with cold WSB, but can be recovered by prolonged extraction with hot WSB(14). The corresponding lipids in maize starch are free fatty acids and lysophospholipids (Table II). However, a small proportion of the free fatty acids could be extracted from washed maize starches with cold WSB, which indicates that these fatty acids are either nonstarch lipids adsorbed onto the surfaces of the starch granules, or they are leaky starch lipids from the peripheral regions of the granules (in which case perhaps there ought to be some leaky lysophospholipids too). The concept of adsorbed, nonstarch free fatty acids is supported by the higher ratio of free fatty acids: lysophospholipids in the pure (washed) starches which had not been exposed to cold WSB, and by the fact that free fatty acids with almost no lysophospholipids were found in pure waxy maize starch but not in the corresponding starch fraction of the endosperm (Table II).

# Pericarp and Tip Cap Lipids

Pericarp lipids contained large amounts of unsaponifiable matter (Table II), which was mostly sterols and ali-

		•	ALLIY DITTELE					ļ		1	TU-11 Marke						Ϋ́.	WaNy maize			
N	Endo	Endosperm							Endosperm						н   	Endosperm					
Lipid + aleurone	Nonstarch Nonstarch + aleurone - aleurone	Aleurone (by difference)	Starch	Pure starch	Germ Pericatp	tp Tip cap		2.	Nonstarch - aleurone	Starch	Pure starch	Germ	Pericarp	Tip cap	Nonstarch + aleurone	Nonstarch - aleurone	Starch	Pure starch	Germ	Pericarp	Tip cap
Steryl ester		29	5	6				61	14	4		818	120	763			-	-	100	46	101
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N-acyl phosphatidyl ethanolamine trace	5		i	1	73 5			3		I	I	110	Ξ	15	-	-		' I	11	v	i
N-acyl lysophosphatidyl ethanolamine	, 2		1	I	1			-		1	ł		-		-	~	ł	I	-	` <b>~</b> `	
Diphosphatidyl glycerol	•		ì	I				1		I	I	8	~ ~-				1	I	90		
Phosphatidyl glycerol	6	9	ł	1	19 4,			-		I	I	36	~		trace	trace	I	I	27	, 7	~
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	31 8	23	I	ł				3		I	I	748	80	41	-	-	ł	I	578	5	
Phosphatidyl inositol	9 2	L	I	1	229 3					I	I	247	7	32			I	I	397	4	15
Phosphatidic acid	2 3	6	I	I				-		I	I	<i>LL</i>	2	24	trace	trace	I	I	59	6	1
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ids		1049	479	704				.9	242	316	403	39359	320	1924	717	426	70	135	34276	229	1914
		- 16	64	48				3	38	34	53	809	27	177	18	91	5	6	1038	11	162
		70	191	212				02	36	248	257	1453	42	185	5	ŝ	ŝ	6	1363	12	52
Unsaponifiable lipids Total lipids 1888	47 21 188 759	26 1129	29 763	67 1031	9095 2143 47272 2460			711	32 348	- 598	- 683	1916 43537	451 840	305 2591	86 826	55 502	° 8	18	2550 39227	936 1203	407 2535

Includes lysophosphatidyl serine

		ļ	Amylomaize	naize					LG-11 maize	naize					Waxy maize	iize		
	Endosperm	Jerm					Endo	Endosperm					Endosperm	perm				
Lipid	Nonstarch	Starch	Germ	Pericarp	Tip cap	Total	Nonstarch	Starch	Germ	Pericarp	Tip cap	Total	Nonstarch	Starch	Germ	Pericarp	Tip cap	Total
Steryl ester	98	10	168	12	5	293	105	~	173	13	6	308	74	1	117	~	5	211
Triglyceride	1671	27	12255	23	81	14057	739	17	7806	17	40	8619	619	76	10147	19	54	10015
Diglyceride	118	7	751	9	s	887	70	9	263	, m	i u	345	52	00	161	m	, œ	205
Free fatty acid	1221	831	259	7	11	2329	286	624	64	T	12	987	913	69	73	11	18	1084
Monoglyceride	35a	16	63a	1a	2a	117 <sup>a</sup>	15a	23	83	tracea	2a	48	19a	÷	38a	2a	2a	643
Estd. steryl glycoside	28	15	96	.3	2	144	14	14	15		7	46	25	e	124	7	ę	157
Monogalactosyl diglyceride	<b>1</b> 20		<b>1</b> 65	~	1	( <sub>170</sub>	ر <sub>عد</sub> (	1,1	( <i>11</i>	1	I	( <sup>40</sup>		<u>~</u>	< <<		÷	~
Monogalactosyl monoglyceride	-	41	2		1	147	\ <del>7</del> \	7 >	175	trace	1	<i>c</i> )		2			<u>ئ</u>	oc >
Digalactosyl diglyceride	18		48	1	1	68	25	61	92	trace	2	138		1	49			52
Digalactosyl monoglyceride	24	63	79	2	1	169	25	17	36	-4	-	80		ŝ	92		1	96
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Diphosphatidyl glycerol	· ~	1			-	~			t 			~ ~	7~	ļ	~	LTACE		
Phosphatidyl glycerol	11	Į	7	~	1	20	-		8			6	-	ł	8	trace	trace	
Phosphatidyl ethanolamine	15	ł	65	ī		81	2		41		-	44	4	I	15	trace	trace	17
Phosphatidyl choline	58	ł	140	1	2	201	7		158	1	1	167	ŝ	1	178	1	trace	182
Phosphatidyl inositol	17	ł	86	1	1	105	9		52		1	59		I	122		1	123
Phosphatidic acid	22	1	124	1	-	148	2		16	trace	1	19	-	1	18	1	-	21
Lysopnospnatidyl giycerol I veorheerhetidyl ethenolemine	7b	1.	ų,	dara ta	ų	ر مارد	ť,	6 ;		trace	<u>ب</u>	وأز		C1 (	47	d		7 de
Lysophosphatidyl choline	54	308		1 I	l	367	36	472 472	\$	trace	trace <sup>0</sup>	514	-	15-	è e	trace		36
Lysophosphatidyl inositol <sup>c</sup>		L			1	~	1	17	•		•	18	¢	•	6			°, 6
Nonpolar lipids	3143	891	13496	49	104	17683	1215	678	8314	34	99	10307	1650	163	10536	43	87	17479
Glycolipids	90	119	288	7	9	510	60	73	170	m	20	343	25	10	320	2	; •	363
Phospholipids	188	353	456	9	6	1012	63	533	307	. 7	4	912	12	11	419	ı ۳	5 2	447
Unsaponifiable lipids	88	54	3393	421	65	4021	155	١	405	48	10	618	198	19	784	175	22	1198
Fotal lipids	3509	1417	17622	102	101													

<sup>a</sup>Includes 6-0-acyl monogalactosyl diglyceride. <sup>b</sup>Includes phosphatidyl serine. <sup>c</sup>Includes lysophosphatidyl serine.

TABLE III

phatic alcohols. The acyl lipids were fairly constant in composition, consisting of triglycerides, steryl esters, free fatty acids and diglycerides. The small amounts of acyl lipids in the senesced pericarp appear to be residues of lipids from early stages of kernel development.

The tip cap lipids contained substantial proportions of nonpolar lipids and unsaponifiable matter (approximately equal amounts of sterols and aliphatic alcohols), together with glycolipids and phospholipids. The tip cap lipids thus resemble pericarp lipids in some respects, while having some of the features of viable tissue such as germ and aleurone.

#### Whole Kernel Lipids

The contribution of lipids in each part of the kernel to the whole kernel lipids is shown in Table III. The whole kernel lipids consisted of 76-85% nonpolar lipids, 2-3% glycolipids, and 4-9% phospholipids, and their compositions fall within the ranges reported by Weber (23).

Apart from free fatty acids and monoglycerides which are in endosperm, the various lipid classes are mostly in the germ, and the whole kernel lipids are therefore similar to the germ lipids. In this respect maize is entirely different from wheat (22) and probably also most other cereals except sorghum and millet.

#### Fatty Acids in Lipids

The fatty acid compositions of most lipids were determined in the course of the work, but are not included in this paper (details can be had on application to the authors). For most purposes the data in Table I provide sufficient information, and they show that all three types of maize had fatty acid compositions within the normal ranges (1,6). Germ lipids (and whole kernel lipids) contained significantly more linoleate than other parts of the kernel.

## DISCUSSION

To the best of our knowledge, the quantitative distribution of all the major acyl lipids throughout a cereal grain has not been described before. The results presented in this paper are consistent with previous analyses of total lipids and fatty acids (2-10) and with what is known about the composition of lipids in the basic structural parts of cereal grains (1).

Our data for lipid classes in the whole kernels of LG-11 (Table III) agree well with Weber's analyses of hybrid 51 at maturity (23,24). However, Weber's data did not reveal the low levels of lipid in endosperm nonstarch fraction and in pericarp, nor the fact that they contained so little polar lipids.

We believe that the lipids in these tissues are remnants of greater quantities of lipids which functioned during kernel

development and were then largely degraded during pericarp senescence and the drying out of the endosperm. In a subsequent study of lipids in developing maize kernels, we obtained data which support this view, and we will publish the results in due course.

In contrast to the above remarks, we consider that free fatty acids and lysophospholipids in maize starches are normal monoacyl lipid components of these starches which may occur as amylose inclusion complexes within the granules, as appears to be the case in other cereals.

#### ACKNOWLDGMENT

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