

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

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## 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

TABLE I

Total Lipid Content, as Fatty Acid Methyl Esters, of Dissected Maize Kernels

|                                        | Percentage of kernel dry wt. | Total lipid (% of dry wt.) |                      | Percentage fatty acid composition <sup>b</sup> |      |      |      |      | Others |  |
|----------------------------------------|------------------------------|----------------------------|----------------------|------------------------------------------------|------|------|------|------|--------|--|
|                                        |                              | Hydrolysis <sup>a</sup>    | Solvent <sup>b</sup> | 16:0                                           | 18:0 | 18:1 | 18:2 | 18:3 |        |  |
| <b>Amylomaize</b>                      |                              |                            |                      |                                                |      |      |      |      |        |  |
| 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  |        |  |
| <b>LG-11</b>                           |                              |                            |                      |                                                |      |      |      |      |        |  |
| 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  |        |  |
| <b>Waxy maize</b>                      |                              |                            |                      |                                                |      |      |      |      |        |  |
| 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  |        |  |

<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 amylo maize 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. Amylo maize 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 phosphorus 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 water-saturated 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 amylo maize 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). Blesin (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 analytical 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 amylo maize 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 amylo maize. Scanning electron micrographs showed that the aleurone cells in LG-11 and waxy maize were isodiametric, while those in amylo maize were radially elongated and approximately twice as large. Thus, it would be reasonable to attribute the greater aleurone lipid content of amylo maize to its larger proportion of aleurone cells, without necessarily invoking a much higher percentage of lipid within aleurone cells.

Amylo maize 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 < 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: amylo maize = 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%. Amylo maize was



TABLE III  
Distribution of Lipids in Amlyomaize, LG-11 Maize and Waxy Maize Kernels ( $\mu\text{g}$  Lipid/Kernel)

| Lipid                                  | Amlyomaize |        |       |          |         |       | LG-11 maize |        |      |          |         |       | Waxy maize |        |       |          |         |       |
|----------------------------------------|------------|--------|-------|----------|---------|-------|-------------|--------|------|----------|---------|-------|------------|--------|-------|----------|---------|-------|
|                                        | Endosperm  |        |       | Total    |         |       | Endosperm   |        |      | Total    |         |       | Endosperm  |        |       | Total    |         |       |
|                                        | 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         | 8      | 173  | 13       | 9       | 308   | 74         | 7      | 117   | 8        | 5       | 211   |
| Triglyceride                           | 1671       | 27     | 12255 | 23       | 81      | 14057 | 739         | 17     | 7806 | 17       | 40      | 8619  | 619        | 76     | 10147 | 19       | 54      | 10915 |
| Diglyceride                            | 118        | 7      | 751   | 6        | 5       | 887   | 70          | 6      | 263  | 3        | 3       | 345   | 25         | 8      | 161   | 3        | 8       | 205   |
| Free fatty acid                        | 1221       | 831    | 259   | 7        | 11      | 2329  | 286         | 624    | 64   | 1        | 12      | 987   | 913        | 69     | 73    | 11       | 18      | 1084  |
| Monoglyceride                          | 35a        | 16     | 63a   | 1a       | 2a      | 117a  | 15a         | 23     | 8a   | tracea   | 2a      | 48    | 19a        | 3      | 38a   | 2a       | 2a      | 64a   |
| Estd. steryl glycoside                 | 28         | 15     | 96    | 3        | 2       | 144   | 14          | 14     | 15   | 1        | 2       | 46    | 25         | 3      | 124   | 2        | 3       | 157   |
| Monogalactosyl diglyceride             | 20         | 41     | 65    | 1        | 1       | 129   | 26          | 23     | 27   | trace    | 1       | 79    | 2          | 2      | 55    | 1        | 1       | 58    |
| Monogalactosyl monoglyceride           | 18         |        | 48    | 1        | 1       | 68    | 25          | 19     | 92   | trace    | 2       | 138   | 2          | 2      | 49    | 1        | 1       | 52    |
| Digalactosyl diglyceride               | 24         | 63     | 79    | 2        | 1       | 169   | 25          | 17     | 36   | 1        | 1       | 80    | 3          | 3      | 92    | 1        | 1       | 96    |
| Digalactosyl monoglyceride             | 1          |        | 27    | 1        | 1       | 30    | 6           | 6      | 23   | 1        | 1       | 31    | 2          | 2      | 36    | 1        | 1       | 39    |
| N-acyl phosphatidyl ethanolamine       | 3          |        |       |          | trace   | 3     | 1           | 1      | 4    | trace    |         | 5     | 2          | 2      | 9     | trace    | trace   | 11    |
| N-acyl lysophosphatidyl ethanolamine   | 11         |        | 7     | 1        | 1       | 20    | 1           | 1      | 8    |          |         | 9     | 1          | 1      | 8     | trace    | trace   | 9     |
| Diphosphatidyl glycerol                | 15         |        | 65    | 1        | 1       | 81    | 2           | 2      | 41   | 1        | 1       | 44    | 2          | 2      | 15    | trace    | trace   | 17    |
| Phosphatidyl glycerol                  | 58         |        | 140   | 1        | 2       | 201   | 7           | 7      | 158  | 1        | 1       | 167   | 3          | 3      | 178   | 1        | 1       | 182   |
| Phosphatidyl ethanolamine              | 17         |        | 86    | 1        | 1       | 105   | 6           | 6      | 52   | 1        | 1       | 59    | 1          | 1      | 122   | 1        | 1       | 123   |
| Phosphatidyl choline                   | 22         |        | 124   | 1        | 1       | 148   | 2           | 2      | 16   | trace    | 1       | 19    | 1          | 1      | 18    | 1        | 1       | 21    |
| Phosphatidyl inositol                  |            |        |       |          |         |       |             |        |      |          |         |       |            |        |       |          |         |       |
| Phosphatidic acid                      |            |        |       |          |         |       |             |        |      |          |         |       |            |        |       |          |         |       |
| Lysophosphatidyl glycerol              | 7b         | 31     | 4b    | traceb   | traceb  | 42b   | 2b          | 9      | 35   | traceb   | traceb  | 37b   | 2          | 2      | 6b    | traceb   | traceb  | 8b    |
| Lysophosphatidyl ethanolamine          | 54         | 308    | 3     | 1        | 1       | 367   | 36          | 17     | 5    | trace    | 1       | 514   | 1          | 7      | 18    | trace    | trace   | 26    |
| Lysophosphatidyl choline               |            |        |       |          |         |       |             |        |      |          |         |       |            |        |       |          |         |       |
| Lysophosphatidyl inositol <sup>c</sup> |            |        |       |          |         |       |             |        |      |          |         |       |            |        |       |          |         |       |
| Nonpolar lipids                        | 3143       | 891    | 13496 | 49       | 104     | 17683 | 1215        | 678    | 8314 | 34       | 66      | 10307 | 1650       | 163    | 10536 | 43       | 87      | 12479 |
| Glycolipids                            | 90         | 119    | 288   | 7        | 6       | 510   | 90          | 73     | 170  | 3        | 7       | 343   | 25         | 10     | 320   | 2        | 6       | 363   |
| Phospholipids                          | 188        | 353    | 456   | 6        | 9       | 1012  | 63          | 533    | 307  | 2        | 7       | 912   | 12         | 11     | 419   | 3        | 2       | 447   |
| Unsaponifiable lipids                  | 88         | 54     | 3393  | 421      | 65      | 4021  | 155         | —      | 405  | 48       | 10      | 618   | 198        | 19     | 784   | 175      | 22      | 1198  |
| Total lipids                           | 3509       | 1417   | 17633 | 483      | 184     | 23226 | 1523        | 1284   | 9196 | 87       | 90      | 12180 | 1885       | 203    | 12059 | 223      | 117     | 14487 |

<sup>a</sup>Includes 6-0-acyl monogalactosyl diglyceride.

<sup>b</sup>Includes phosphatidyl serine.

<sup>c</sup>Includes lysophosphatidyl serine.

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.

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