

# Phospholipids of Six Seed Oils of Malvaceae

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The phospholipid fractions of six seed oils of the Malvaceae family—*Gossypium barbadense* (Egyptian cotton), *Hibiscus cannabinus* (kenaf), *Hibiscus sabdarifa* (roselle), two varieties of *Hibiscus esculentus* (okra) and *Althea rosea* (ketmia, hollyhock or Egyptian hemp)—have been isolated by silicic acid chromatography. Thin-layer chromatography of these phospholipid fractions revealed that the common phosphatides were cephalins, lecithin (phosphatidylcholine) and some of their lysoforms. There were also some nonpolar constituents, especially phosphatidic acid. Phospholipid fatty acids were prepared by transesterification. Different proportions of three common fatty acids, palmitic, oleic and linoleic, have been found in the six glycerophospholipids.

**KEY WORDS:** Composition, fatty acids, lipase, Malvaceae, oils, seeds, triacylglycerols.

Although phosphatides are the most investigated constituents of the complex lipids, the literature data is relatively limited. However, cottonseed oil phosphatides have been thoroughly studied by El-Nockrashy *et al.* (1,2) using silicic acid column and thin-layer chromatography. Also they determined the fatty acid composition of both individual and total phosphatides. Other authors have also studied cottonseed oil phospholipids, especially their fatty acids (3–6). A few articles dealt with the phosphatides of kenaf seed oil (7–9).

## EXPERIMENTAL

**Lipid extraction.** Seeds of Egyptian cotton, kenaf, roselle, okra (two varieties) and ketmia (hollyhock), were purchased from a local seed and herb store. The oil was extracted by chloroform/methanol, 2:1, at room temperature (10).

**Preparation of phospholipid fractions.** The phospholipid fractions were prepared by shaking the oil with chloroform silicic acid (100-mesh Mallinckrodt, St. Louis, MO) slurry. The filtered residue was re-extracted with methanol. The dried methanol residue constitutes the phospholipid fraction of the oil (11–13).

**Thin-layer chromatography.** The six phospholipid fractions were spotted against authentic samples on silica gel G plates. The plates were developed with chloroform/methanol/water (65:24:4, v/v/v) (14). The spots were visualized by iodine vapors.

**Gas liquid chromatography.** A portion of each of the phospholipid fractions was converted to methyl esters by transesterification with methanol/HCl (15). The methyl esters of the phospholipids were analyzed by gas liquid chromatography.

## RESULTS AND DISCUSSION

The oil and phospholipid contents are shown in Table 1. Oil percentages ranged from 10.7 for ketmia seed to

TABLE 1

Oil and Phospholipid Percents

	Cotton	Kenaf	Roselle	Okra <sup>a</sup>	Okra <sup>b</sup>	Ketmia (hollyhock)
Oil	19.5	21.1	20.0	20.5	22.1	10.7
PL <sup>c</sup>	1.9	1.3	1.7	1.3	1.2	1.3

<sup>a</sup>*Hibiscus esculentus* var. *baladi*.

<sup>b</sup>*Hibiscus esculentus* var. *romi*.

<sup>c</sup>Phospholipid.

around 20 for each of the other five seeds. The average oil content for the six seeds was ca. 19%, while it was ca. 15% for other *Hibiscus* species (16,17). Phospholipid contents ranged from 1.9% for cotton (2) to 1.2% for okra (*Hibiscus esculentus* var. *romi*) oils.

The qualitative composition of the phospholipid fractions is shown in Figure 1. Combination of silica gel G and chloroform/methanol/water system proved to be an excellent method for fractionation of the glycerophospholipids (11–14,18). Three nonpolar acidic phosphatides were present in the six samples. Phosphatidic acid ( $R_f$  0.95), which is the precursor of all other glycerophospholipids and triacylglycerols (11,12), was abundant in all samples. Two other components were less abundant in five samples and were absent in kenaf oil. These were phosphatidylglycerol ( $R_f$  0.90) and polyglycerophosphatide ( $R_f$  0.85). Some authors concluded that these two spots in cotton polyglycerophospholipid were cerebrosides (1,19). The well represented cephalin constituents—phosphatidylethanolamine, phosphatidylserine (not in kenaf) and a minor component, phosphatidylinositol—were present in all samples (1,2,19). They were separated according to polarity and identified by comparison with authentic samples,  $R_f$  0.67, 0.40 and 0.04. Phosphatidylcholine (lecithin), the most common plant phosphatide (18), constituted the major lipid component in all samples and had an  $R_f$  0.52. Four polar phospholipid constituents with  $R_f$  0.26, 0.23, 0.15 and 0.08 were identified as sphingolipid, lysophosphatidylethanolamine, lysolecithin and N-lysophosphatidylethanolamine, respectively. Each was present in all samples, but in very small amounts. Some of these polar lipids were previously isolated from cotton (1) and kenaf (4,7,19) oils.

On the other hand, the fatty acid composition (Table 2) of the different phosphatidylglycerol fractions of the six oil samples shows the same acids as those of their triacylglycerols (20), but with some variations in their proportions. This is in agreement with the previous assumption that in vegetable oils, the fatty acids in the phosphatides are similar to those in the triglycerides (21). However, Persmark later found that the proportions and kinds of fatty acids in the phosphatides of rapeseed are different from those in the triacylglycerol oils (22). The same was found in umbelliferous seed oils (23). On the basis of limited data, one cannot draw a conclusion concerning the identity and proportions of fatty acids in vegetable oil

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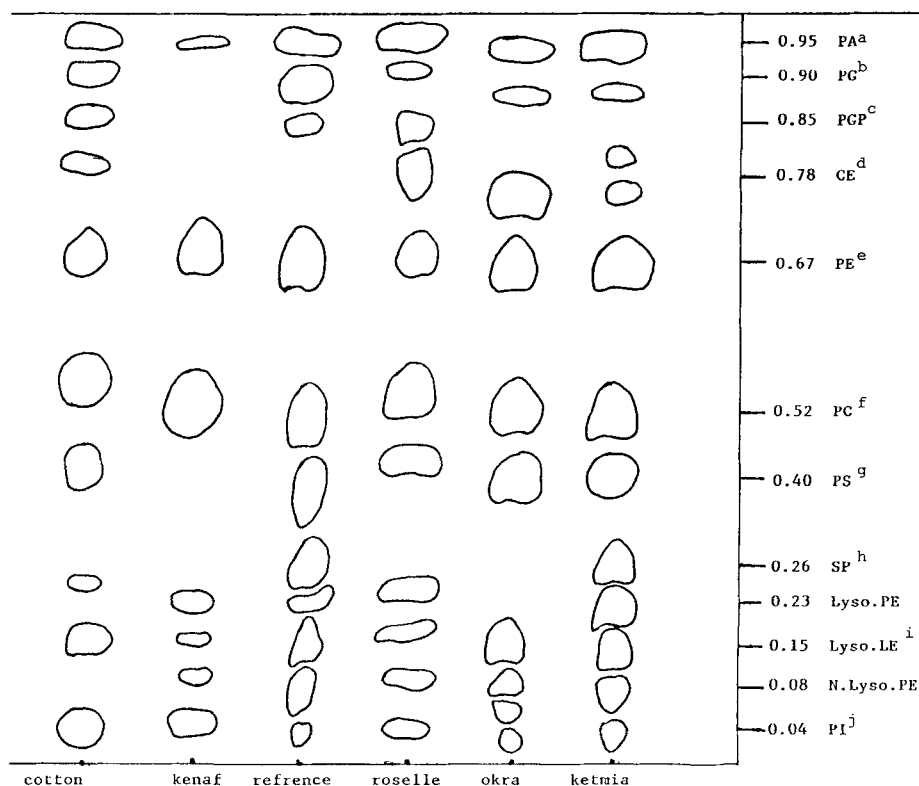


FIG. 1. Thin-layer chromatography of phospholipids: <sup>a</sup>phosphatidic acid; <sup>b</sup>phosphatidylglycerol; <sup>c</sup>polyglycerolphosphate; <sup>d</sup>cerebroside; <sup>e</sup>phosphatidylethanolamine; <sup>f</sup>phosphatidylcholine (lecithin, LE); <sup>g</sup>phosphatidylserine; <sup>h</sup>sphingolipid; <sup>i</sup>lysolecithin; and <sup>j</sup>phosphatidylinositol.

TABLE 2

Fatty Acids of Whole TAGs<sup>a</sup> and PL<sup>b</sup> Fractions

Fatty acid	Cotton		Kenaf		Roselle		Okra <sup>c</sup>		Okra <sup>d</sup>		Ketmia (hollyhock)	
	TAGs	PL	TAGs	PL	TAGs	PL	TAGs	PL	TAGs	PL	TAGs	PL
< C <sub>16</sub>	0.7	3.6	—	—	0.3	—	0.8	—	—	2.1	0.5	1.5
C <sub>16:0</sub>	29.8	35.6	35.4	36.5	34.1	39.7	41.3	40.3	37.9	27.2	34.5	28.5
C <sub>16:1</sub>	—	1.4	—	—	—	—	—	—	—	1.4	—	—
C <sub>18:0</sub>	0.7	0.8	—	—	2.2	—	1.0	—	—	1.6	1.0	2.7
C <sub>18:1</sub>	16.5	18.0	25.9	31.7	33.4	31.3	28.2	24.7	21.5	35.2	21.0	18.6
C <sub>18:2</sub>	49.8	40.6	36.0	31.8	28.8	29.0	22.5	35.0	35.5	32.5	37.8	48.7
C <sub>20:1</sub>	2.5	—	2.7	—	1.2	—	6.2	—	5.1	—	5.2	—

<sup>a</sup>Triacylglycerols (20).

<sup>b</sup>Phospholipids.

<sup>c</sup>*Hibiscus esculentus* var. *baladi*.

<sup>d</sup>*Hibiscus esculentus* var. *romi*.

phosphatides or their triglycerides. For a better understanding of their chemical and biochemical applications, further studies of the distribution of fatty acids in both glycerophospholipids and triacylglycerols are needed.

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