

## Chapter Four

### Cottonseed Lecithin

#### Abstract

Industrial lecithin can be fractionated from cottonseed as phospholipids and glycolipids after neutral lipids and protein-containing contaminants are removed. The purity and special properties of the lecithin can be improved by a number of solvent extraction and precipitation procedures. Studies are determining the role of the phospholipids of lecithin in the synthesis of triglycerides in maturing cottonseed, and the molecular basis of the functionality of food ingredients. Lecithin, having both polar and non-polar groups, has high surface activity and is reactive with both oil and protein, making it an excellent emulsifying agent in food systems. Lecithin also slows autoxidation and enzyme hydrolysis of fats. Cottonseed lecithin is low in linolenic acid and slows flavor and color deterioration processes when blended with other vegetable oils. Gossypol binds to lecithin during oil extraction from glanded cottonseed, economically negating the use of these phospholipids as a commercial source. New cultivars producing glandless or gossypol-free cottonseed, now being grown in southwestern and western United States, have potential as commercial sources of edible lecithin.

#### Introduction

Phospholipids contribute to transport and metabolic reactions of nutritive substances in living systems (1-5). Lecithin is the commercial or popular name for the naturally occurring mixture of several phospholipids, obtained mainly at this time from soybeans, including lecithin (phosphatidylcholine) and cephalin (phosphatidylethanolamine, phosphatidylserine, and phos-

phatidylinositol) (Fig. 1; 5, 6). Diphosphatidylglycerol, lysophosphatidylcholine and a group of minor and unidentified polar lipids are present in small amounts (Fig. 2; 5, 7). Phospholipids are used as emulsifiers and antioxidants in many food systems (5, 8, 9). These applications generally take advantage of the primary property of phospholipids (polar and nonpolar groups contained within the molecular structures) to lower surface tension of liquid and dry substances into which they are incorporated, and to make homogeneous systems out of mixtures such as oil and protein, and as immiscible liquid phases.

PHOSPHOLIPID	STRUCTURE: $\alpha$ -FORM	$\beta$ -FORM	TRIVIAL NAME
PHOSPHATIDYL CHOLINE	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-R}' \\   \\ \text{CH}_2\text{-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-N}^+(\text{CH}_3)_3 \end{array}$	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-N}^+(\text{CH}_3)_3 \\   \\ \text{CH}_2\text{-O-R}' \end{array}$	LECITHIN
PHOSPHATIDYL ETHANOLAMINE	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-R}' \\   \\ \text{CH}_2\text{-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-NH}_3^+ \end{array}$	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-NH}_3^+ \\   \\ \text{CH}_2\text{-O-R}' \end{array}$	CEPHALIP
N-ACYL PHOSPHATIDYL ETHANOLAMINE	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-R}' \\   \\ \text{CH}_2\text{-O-P(=O)(OH)-O-CH}_2\text{-CH}_2\text{-NH-R}'' \end{array}$	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-P(=O)(OH)-O-CH}_2\text{-CH}_2\text{NH-R}' \\   \\ \text{CH}_2\text{-O-R}' \end{array}$	N.A.
PHOSPHATIDYL SERINE	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-R}' \\   \\ \text{CH}_2\text{-O-P(=O)(OH)-O-CH}_2\text{-CH}_2\text{-C(=O)-NH}_3^+ \end{array}$	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-P(=O)(OH)-O-CH}_2\text{-CH-C(=O)-NH}_3^+ \\   \\ \text{CH}_2\text{-O-R}' \end{array}$	CEPHALIN
PHOSPHATIDYL INOSITOL	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-R}' \\   \\ \text{CH}_2\text{-O-P(=O)(OH)-O-C}_6\text{H}_8\text{-(OH)}_6 \end{array}$	$\begin{array}{c} \text{CH}_2\text{-O-R} \\   \\ \text{CH-O-P(=O)(OH)-O-C}_6\text{H}_8\text{-(OH)}_6 \\   \\ \text{CH}_2\text{-O-R}' \end{array}$	CEPHALIN

Fig. 4-1. Structures of phospholipids. R, R<sup>1,11</sup> = various fatty acids; N.A. = not available. (5)

The phospholipids phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid were shown to contribute to antioxidant properties in

*Cottonseed Lecithin*

PHOSPHOLIPID	STRUCTURE: $\alpha$ -FORM	TRIVIAL NAME
SPHINGOMYELIN	$  \begin{array}{c}  \text{CH}_2\text{-O-CH=CH-(CH}_2\text{)}_{12}\text{-CH}_3 \\    \\  \text{CH-O-R} \\    \\  \text{CH}_2\text{-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-N}^+\text{(CH}_3\text{)}_3  \end{array}  $	N. A.
PHOSPHATIDYL GLYCEROL	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \quad \text{CH}_2\text{-OH} \\    \quad \quad   \\  \text{CH-O-R}' \quad \text{CH-OH} \\    \quad \quad   \\  \text{CH}_2\text{-O-P(=O)(OH)-O-CH}_2  \end{array}  $	N. A.
DIPHOSPHATIDYL GLYCEROL	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \quad \text{CH}_2\text{-O-P(OH)(O-CH}_2\text{)-O-CH}_2 \\    \quad \quad   \quad \quad   \\  \text{CH-O-R}' \quad \text{CH-OH} \quad \text{CH-O-R}'' \\    \quad \quad   \quad \quad   \\  \text{CH}_2\text{-O-P(=O)(OH)-O-CH}_2 \quad \text{CH}_2\text{-O-R}'''  \end{array}  $	CARDIOLIPIN
PHOSPHATIDIC ACID	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \\    \\  \text{CH-O-R}' \\    \\  \text{CH}_2\text{-O-P(=O)(OH)}  \end{array}  $	N. A.
LYSOPHOSPHATIDYL CHOLINE	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \\    \\  \text{CH-OH} \\    \\  \text{CH}_2\text{-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-N}^+\text{(CH}_3\text{)}_3  \end{array}  $	LYSOLECITHIN
LYSOPHOSPHATIDYL ETHANOLAMINE	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \\    \\  \text{CH-OH} \\    \\  \text{CH}_2\text{-O-P(=O)(O}^-\text{)-O-CH}_2\text{-CH}_2\text{-NH}_3^+  \end{array}  $	LYSOCEPHALIN
LYSOPHOSPHATIDYL SERINE	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \\    \\  \text{CH-OH} \\    \\  \text{CH}_2\text{-O-P(=O)(OH)-O-CH}_2\text{-CH(NH}_3^+\text{)-C(=O)-O}^-  \end{array}  $	N. A.
LYSOPHOSPHATIDYL INOSITOL	$  \begin{array}{c}  \text{CH}_2\text{-O-R} \\    \\  \text{CH-OH} \\    \\  \text{CH}_2\text{-O-P(=O)(OH)-O-C}_6\text{H}_8\text{(OH)}_5  \end{array}  $	N. A.

Fig. 4-2. Structures of minor phospholipids. R, R<sup>I,II,III</sup> = various fatty acids; N.A. = not available. (5)

stored foods (10–12). Phosphatidylethanolamine gives the best protection against oxidation of unsaturated fatty acids. Addition of phosphatidylethanolamine and phosphatidylcholine reduced lipolysis, probably by interacting with the lipase system. The fatty acid portion of the phospholipid molecules played no role in protecting against food oxidation, although a lack of linolenic acid in cottonseed lecithin contributed to storage stability.

### Phospholipid Extraction and Composition

During the 1930s and 1940s, cottonseed phospholipids were, to a small extent, commercially available (13). Most of the phospholipids were in the nonoil materials (1.0–2.0%) separated from hydraulically pressed oil by alkali refining or water washing. The phospholipids could also be extracted from the nonoil material with either ether, petroleum naphtha or benzene. They were shown to be relatively insoluble in acetone, and once purified could be further separated into alcohol-soluble lecithins and alcohol-insoluble cephalins.

From a commercial cottonseed product, Olcott (14) obtained a bright yellow-colored powdery solid of purified phospholipid (phosphorus, 2.9%; nitrogen, 1.2%) and noted that it was relatively storage-stable. The fatty acid composition of these phospholipids from cottonseed was palmitic acid, 17.3%; stearic acid, 7.3%; arachidic acid, 2.8%; palmitoleic acid, 1.5%; oleic acid, 20.3%; linoleic acid, 44.4%; and unsaturated C<sub>20–22</sub> fatty acids, 6.4%. The absence of the triunsaturated linolenic acid contributed to the storage stability of cottonseed phospholipids.

Since highly pure lecithin with equivalent fatty acids as crude preparations would yield the considerably different analysis of about 4.0% phosphorus, 1.8% nitrogen, 70% fatty acid and no ash, Woolley (15) suggested that the phospholipid fraction of cottonseed oil did not consist predominantly of lecithins and cephalins, but contained others like “lipositol” found in soybean oil. In lipositol (phosphatidylinositol; Figs. 1, 2), the polyhydric alcohol with which the fatty acids are esterified, was suggested to consist of the cyclic alcohol inositol. Also esterified with the alcohol, or otherwise combined, are phosphoric acid, ethanolamine tartrate and galactose.

Lishkevich (16) found that the phospholipid content of various oilseeds was highest in cottonseed, followed by soybean, sunflower, flax, castor bean and peanut seed. He separated cottonseed phospholipids into three fractions including (a) acetone soluble, 16.5%, of which 46.2% was lecithin, and 53.8% was cephalin; (b) acetone insoluble, 76.5%, of which 53.2–59.4% was lecithin, and 40.6–46.8% was cephalin; and (c) benzene soluble, 7.0%, which

was almost entirely lecithin. In other studies, associated impurities were removed from the phosphorus-containing residues by washing them with acidified water (<pH 3.0) or with 10% sodium chloride solution (17-21). The acetone insoluble portion was shown to contain bound gossypol (20).

The method of cottonseed crushing and extraction affects the phospholipid content in oil (22-25). Hydraulically pressed oils were richer in acetone insoluble materials, including phospholipids, than those extracted with either screw prepress-solvent or solvent methods (Table I). The greatest percentage of phospholipid recovered was from the solvent extracted oil (Table II). Total phospholipid content was highest in the screw prepress-solvent extracted oil. Phospholipids (% of total) extracted varied little among extraction methods and included phosphatidylinositol, lysolecithin, phosphatidylserine and phosphatidylethanolamine. The infra-red (IR) spectrum of lecithin revealed a band at  $5.8 \mu$  corresponding to the carbonyl ester of glycerophosphatide, and  $10.3 \mu$ , a property of phospholipids with choline (Fig. 3; 25). Bands at 6.8, 8.1, 8.6, and 9.1 represent  $\text{CH}_{1-3}$ ,  $\text{P}=\text{O}$ ,  $\text{C}-\text{O}-\text{C}$ , and  $\text{P}-\text{O}-$  groups, respectively. Fatty acid composition of phospholipids from cottonseed oil varied according to the extraction method (Table III). Neutral lipids from cottonseed contained more polyunsaturated fatty acids than the phospholipids; the reverse was true for the saturated fatty acids. Phospholipids extracted by hexane contained the highest percentage of linoleic acid. The highest percentage of oleic acid was present in phospholipids of chloroform-extracted oil. Ether-extracted phospholipids were highest in unsaturated fatty acids.

For comparison, Alter and Gutfinger (26) presented percentages of fatty acids of prepress-solvent and solvent extracted cottonseed neutral and polar lipid fractions (Table IV). The content of saturated fatty acids was significantly higher in the phospholipid fractions than in the corresponding neutral lipid portions. The increase in saturated fatty acids was associated with a corresponding reduction in the dienoic fatty acid, linoleic acid. Overall, neutral lipids from cottonseed contained more polyunsaturated fatty acids than their phospholipids.

Gas liquid chromatographic (GLC) analysis of fatty acid methyl esters derived from total lecithin of cottonseed showed approximately 80% unsaturated fatty acids (27). The fatty acids in the  $\beta$ -position of cottonseed lecithin that are liberated by phospholipase- $\text{A}_2$  hydrolysis consist mainly of oleic (29.5%) and linoleic (61.9%) acids. Cottonseed lecithin also contains 8.6% palmitic acid in the  $\beta$ -position. The  $\alpha$ -position consists mainly of palmitic, oleic and linoleic acids, and a small amount of stearic acid. The unsaturated palmitic and stearic acids make up 60% of the  $\alpha$ -position.

The observations showing that percentage of individual phospholipids in

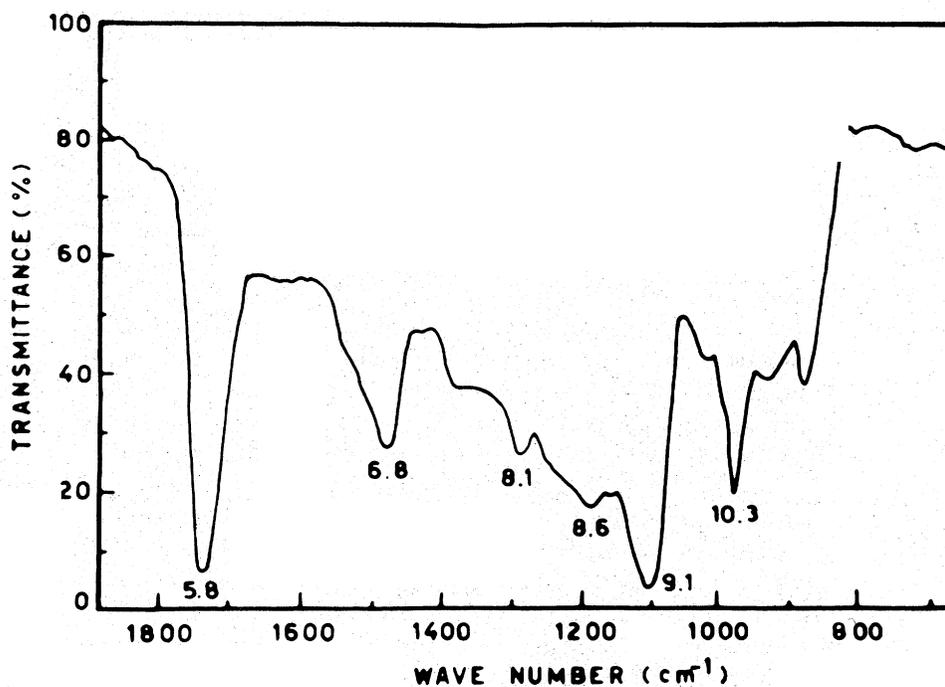


Fig. 4-3. Infra-red spectrum of cottonseed lecithin. (25)

oil varies with the processing technique were emphasized (27, 28). Such differences are less prevalent when the individual phospholipid composition is calculated as percentage of total phospholipids. For example, cooking crushed cottonseed enhances the extraction of phospholipids with oil. In hydraulic and screw press methods, the greater amount of phospholipids remains in the cake than oil. Therefore, solvent extracted oil contains higher phospholipid content than oil from the press procedures. The average amounts of individual components in the phospholipids are 26.3% phosphatidylcholine, 20.2% phosphatidylserine, 17.7% phosphatidylethanolamine, 9.3% phosphatidylinositol, 5.7% lysolecithin and 12.7% unknown substances.

Vijayalakshmi et al. (29) showed that 92% of the cottonseed phospholipids could be eluted from a silicic acid column and contained 33% phosphatidylcholine, 20% phosphatidylethanolamine and 37% phosphatidylinositol. Other phospholipids (8%) consisted of phytoglycolipid, phytosphingosine and phosphatidylserine (Figs. 1, 2). This study also showed that cottonseed phospholipids are rich in simple inositol phospholipids (Fig. 2). The sum of

**TABLE 4-I**  
**Composition (%)<sup>a</sup> of Cottonseed Soapstock Prepared**  
**by Different Oil Extraction Methods (23)**

Analysis	Extraction method		
	Hydraulic press	Screw prepress-solvent	Solvent
Moisture, volatiles	46.03	43.47	44.11
Free fatty acids	0.17	0.27	0.20
Total fatty acids	27.99	28.88	27.68
Neutral oil	10.45	13.67	10.99
Ash	3.05	3.15	3.05
Successive extractions:			
Hexane soluble	0.54	0.64	1.47
Ether soluble	2.68	3.37	3.14
Chloroform soluble	8.07	8.19	8.25
Successive extractions:			
Acetone insoluble			
from hexane soluble	8.73	6.49	4.60
Acetone insoluble			
from ether soluble	15.97	8.78	7.42
Acetone insoluble			
from chloroform soluble	89.99	51.21	47.20

<sup>a</sup>Averages of two lots.

phosphatidylethanolamine and phosphatidylinositol, about 59%, was in fair agreement with values of alcohol-extracted cephalin, at 71% (Fig. 1; 30). The phospholipids contained gossypol, probably bound to phosphatidylethanolamine and phosphatidylinositol (Fig. 4). This interaction was disrupted by conditions of silicic acid chromatography (29), or mildly acidic pH (31).

In summary, the saturated:unsaturated fatty acid ratio of cottonseed phospholipids is approximately 1:2 (Table V; 5, 22). Palmitic acid constitutes 90% of the total saturated fatty acids, while linoleic acid is approximately 80% of the total unsaturated fatty acids. Isolated cottonseed crude phospholipids contain approximately 9.13% total gossypol, some of which is in the free form. Most of the gossypol is associated with phosphatidylethanolamine and phosphatidylserine. Yields of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine are 34.85%, 20.05% and 16.2%, respectively, of the acetone-insoluble residue. Total saturated fatty acid composition is higher in phosphatidylethanolamine than in phosphatidylcholine or phosphatidylserine. Phosphatidylethanolamine has the lowest amount of unsaturated fatty acids of the three phospholipids. Palmitic acid

**TABLE 4-II**  
**Phospholipid Composition<sup>a</sup> (%) of Cottonseed Soapstock**  
**Prepared by Different Oil Extraction Methods (23)**

Phospholipid	Extraction method					
	Hydraulic press		Screw prepress-solvent <sup>b</sup>		Solvent <sup>b</sup>	
	In soap-stock	Of total phospho-lipid	In soap-stock	Of total phospho-lipid	In soap-stock	Of total phospho-lipid
Phosphatidylinositol	0.03	5.91	0.05	5.72	0.35	5.96
Lysolecithin	0.05	9.23	0.07	9.39	0.06	9.25
Phosphatidylserine	0.17	29.92	0.27	28.50	0.21	28.63
Phosphatidylethanolamine	0.15	34.17	0.22	35.24	0.17	35.13
Unknown	0.06	12.61	0.10	12.99	0.08	13.22
% Recovery	—	91.83	—	91.83	—	92.18
Total phospholipid	0.51	—	0.78	—	0.59	—

<sup>a</sup>Averages of two lots.

<sup>b</sup>Hexane.

**TABLE 4-III**  
**Fatty Acid Composition (%) of Cottonseed Soapstock and Phospholipids (23)**

Fatty acid	Hexane soluble soapstock	Phospho-lipid of hexane soluble fraction	Ether soluble soapstock	Phospho-lipid of ether soluble fraction	Chloroform soluble soapstock	Phospho-lipid of chloroform soluble fraction
C <sub>14:0</sub>	0.36	0.24	0.26	0.21	0.31	0.54
C <sub>16:0</sub>	20.68	22.26	20.16	34.26	24.96	10.87
C <sub>18:1</sub>	14.26	16.57	10.47	8.57	9.34	35.33
C <sub>18:2</sub>	64.70	60.93	69.11	56.96	65.39	53.26

is the major saturated fatty acid, and oleic and linoleic acids make up most of the unsaturated fatty acids.

### Varietal Phospholipids

Shustanova et al. (32) presented yields of free and bound lipids of cottonseed from two *Gossypium hirsutum* varieties, S-4727 and S-6029 (Table VI). The lipids were extracted by successively treating full fat meals with a number of

**TABLE 4-IV**  
**Fatty Acids (%) in Phospholipids and Neutral Lipids of Cottonseed Oil (26)**

Fatty acid <sup>a</sup>	Extraction technique			
	Prepress-solvent <sup>b</sup>		Solvent <sup>b</sup>	
	Phospholipids	Neutral lipids	Phospholipids	Neutral lipids
C <sub>14:0</sub>	1.4	1.2	1.4	1.3
C <sub>16:0</sub>	31.5	25.6	30.0	25.3
C <sub>18:0</sub>	4.0	2.4	3.8	2.3
C <sub>18:1</sub>	20.8	18.8	21.0	18.8
C <sub>18:2</sub>	42.3	52.0	43.8	52.3
Saturated fatty acids	36.9	29.2	35.2	28.9
Polyunsaturated fatty acids	42.3	52.0	43.8	52.3

<sup>a</sup>C<sub>16:1</sub>, trace; C<sub>18:3</sub>, none detected.

<sup>b</sup>Hexane.

solvents and fractionating the components by silica gel two-dimensional chromatography. The main components of the phospholipid fraction were identified as phosphatidylcholine (52.0%), phosphatidylinositol (21.8%), and phosphatidylethanolamine (11.4%). Minor components were polyglycerophosphatides (two components, 2.8% and 5.8%), lysophosphatidylcholine (1.7%) and unidentified polar phospholipids (4.5%).

On the whole, the total phospholipid composition was similar for the two cotton varieties (Table VII). Variety S-4727 contained more unsaturated fatty acids than variety S-6029. This was particularly noticeable in the total phospholipid fraction. Thus, the potential exists for breeding for select fatty acid composition in cottonseed phospholipids. The fatty acid compositions of the total phospholipid fraction of both varieties were more saturated than the other lipid extracts; this was due mostly to the high palmitic acid composition. Of the unsaturated fatty acids, linoleic and oleic acids predominated. Overall, the free lipids extracted by acetone contained more oleic acid and less linoleic acid than the petroleum ether extract.

### Minor Phospholipids

Minor phospholipids, cerebroside and phytoglycolipid, are isolated by extensive sequential fractionation with solvent (acetic acid, benzene insoluble), silicic acid column chromatography and preparative thin layer chromatography in separate analyses, (93:7 and 30:70 chloroform:methanol separate cerebroside and phytoglycolipid, respectively) (33). The sugar moieties of

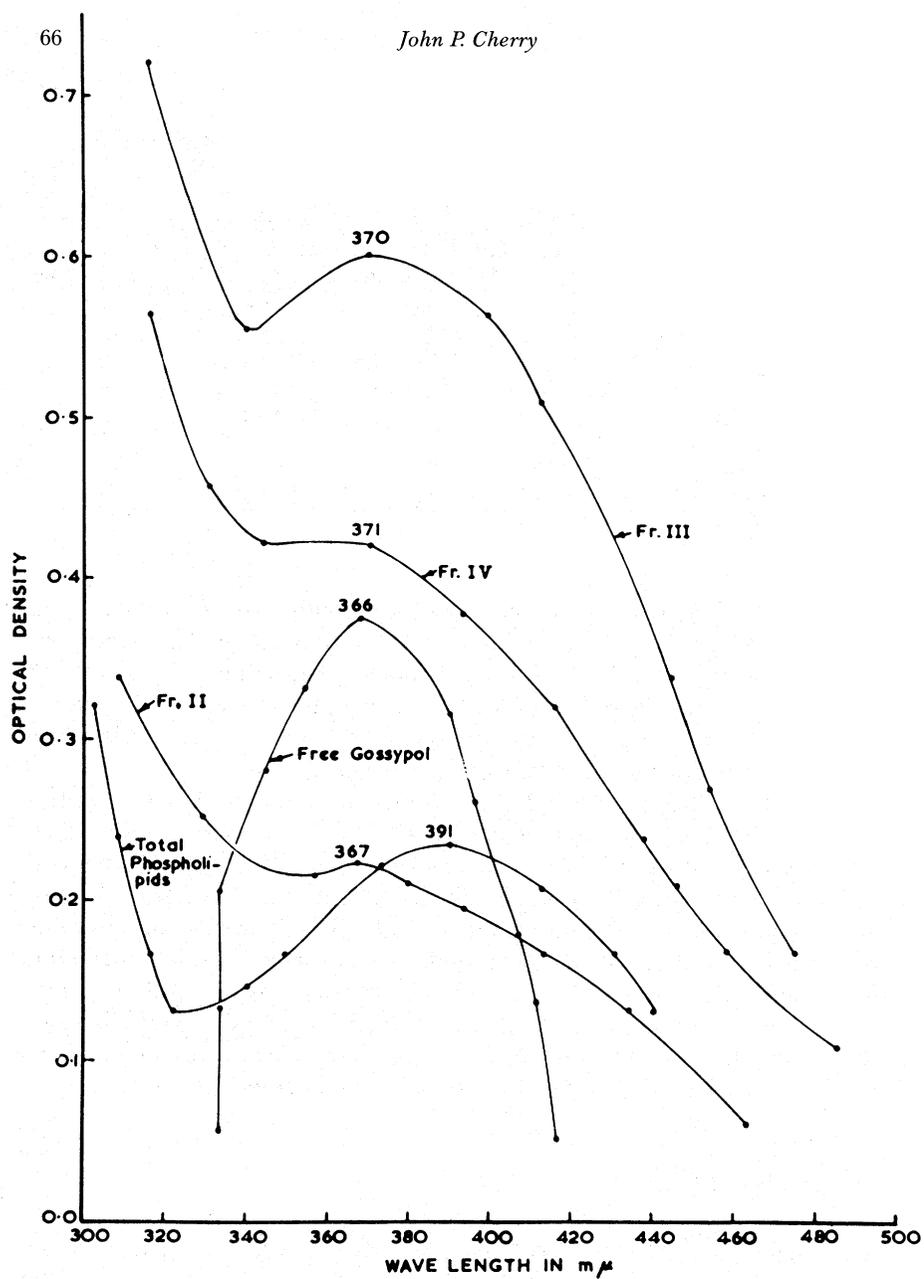


Fig. 4-4. Ultraviolet absorption spectra of total cottonseed phospholipids, column chromatographic fractions and free gossypol; given is the wavelength of  $\lambda$  maximum. Fr. (Fraction) II = glycerides + gossypol; Fr. III = phosphatidylethanolamine + gossypol; Fr. IV = phosphatidylinositol + gossypol. (29)

**TABLE 4-V**  
**Composition of the Acetone-Insoluble Fraction of Cottonseed Oil (5, 22)**

Component	Crude phospholipids <sup>a</sup>	Phosphatidylcholine <sup>b</sup>	Phosphatidylethanolamine <sup>b</sup>	phosphatidylserine <sup>b</sup>
Extracted	2.24	34.85	20.05	16.20
Nitrogen	1.19	1.58	1.34	1.19
Phosphorus	2.49	3.37	2.67	2.21
Total gossypol	9.13	2.34	22.43	19.90
Free gossypol	0.02	2.24	0.05	0.01
Fatty acid				
C <sub>8:0</sub>	—	—	0.4	0.3
C <sub>12:0</sub>	—	—	0.4	0.4
C <sub>14:0</sub>	0.4	0.3	0.4	0.6
C <sub>16:0</sub>	32.9	31.1	33.7	33.3
C <sub>16:1</sub>	0.5	0.3	0.3	0.6
C <sub>18:0</sub>	2.7	2.8	2.2	0.3
C <sub>18:1</sub>	13.6	11.0	11.5	14.4
C <sub>18:2</sub>	50.0	54.0	49.0	50.4
Unknown	—	—	2.3	—
Saturated fatty acids	36.0	34.2	37.1	34.9
Unsaturated fatty acids	64.1	65.3	60.8	65.4

<sup>a</sup>Percentage of crude oil.

<sup>b</sup>Percentage of crude phospholipids.

cerebroside are glucosamine, glucose and rhamnose. Those of phytoglycolipid are glucuronic acid, galactose, glucose and rhamnose. The IR spectra of these components confirm their structures. Specifically, in addition to the bands noted for lecithin (Fig. 3), cerebroside has bands at 6.04 and 6.4  $\mu$  corresponding to amides I and II, which are characteristic of sphingolipids, while phytoglycolipid has a band at 5.7  $\mu$ , typical of glycerophosphatides. These phospholipids contain substantial amounts of hydroxylated fatty acids. Hydroxy:nonhydroxy fatty acid ratios in cerebroside and phytoglycolipid are 1:9 and 1:16, respectively. Oleic acid (C<sub>18:1</sub>) makes up most of the nonhydroxy acids (45.9% and 60.4% for cerebroside and phytoglycolipid, respectively). Nonhydroxy linoleic acid (C<sub>18:2</sub>) is the main polyunsaturated fatty acid, making up only 0.6% of the fatty acids in cerebroside and 10.0% in phytoglycolipid. The total saturated nonhydroxy acids in the minor phospholipids are 53.2% and 19.5%, respectively. The major saturated nonhydroxy acid in cerebroside is C<sub>24:0</sub> (25.6%); others include stearic, palmitic and arachidic acids. No hydroxy C<sub>24</sub> is present in either of the minor phospholipids. Hydroxy C<sub>18:1</sub> is a major fatty acid in both minor phospholipids

TABLE 4-VI  
 Successive Extraction (%) with Various Solvents of Free and Bound Lipids from Seeds of Two Cotton Varieties (32)

Solvent	Extracted compounds	Variety	Yield		Phosphorus content	Distribution of phosphorus	
			Kernel basis	Fraction basis		Kernel basis	Total phosphorus
Petroleum ether	Glycerides, fatty acids, gossypol, etc.	S-4727	31.3	72.4	0.0045	0.0014	2.5
			26.3	72.4	0.0038	0.0010	1.7
Acetone	Gossypol, glycerides, fatty acids, phospholipids, etc.	S-4727	6.1	14.2	0.050	0.003	5.2
			5.0	13.5	0.046	0.002	3.4
Chloroform-methanol (2:1) <sup>a</sup>	Phospholipids, carbohydrates, glycerides, etc.	S-4727	6.1	13.4	1.20	0.0525	92.3
			5.3	14.4	1.12	0.0550	94.9

<sup>a</sup>Best extraction technique.

**TABLE 4-VII**  
**Fatty Acid Composition (%) of Free and Bound Lipids**  
**from Seeds of Two Cotton Varieties (32)**

Fatty acid	Extract					
	Petroleum ether		Acetone		Total phospholipid	
	S-4727	S-6029	S-4727	S-6029	S-4727	S-6029
C <sub>10:0</sub>	0.5	2.2	0.4	1.2	0.6	3.5
C <sub>12:0</sub>	0.4	0.9	0.3	0.6	0.5	0.7
C <sub>14:0</sub>	0.5	0.7	0.5	0.5	0.5	0.5
C <sub>16:0</sub>	24.0	23.4	22.8	23.5	25.4	27.9
C <sub>16:1</sub>	trace	1.3	0.5	1.2	0.7	0.9
C <sub>18:0</sub>	0.7	0.8	1.4	1.1	2.3	0.9
C <sub>18:1</sub>	18.1	13.6	25.4	21.6	17.3	13.2
C <sub>18:2</sub>	54.3	57.1	48.1	50.3	52.0	52.4
C <sub>18:3</sub>	1.5	0	0.7	0	0.7	0
Total saturated	26.1	28.0	25.3	26.9	29.3	33.5
Total unsaturated	73.9	72.0	74.7	73.1	70.7	66.5

(40.9% and 50.7%, respectively). Hydroxy C<sub>18:2</sub> is present in amounts of 9.0% and 10.0%, respectively. Total saturated hydroxy acid composition, mainly stearic acid, is 44.3% and 30.5%, respectively. Cerebroside contains 1.43% N and no P; phytoglycolipid has 1.37% N and 2.68% P. The presence or absence of phosphorus is the major difference of the two minor lipids. Gossypol is not present in either fraction; no available amine groups are present for reaction with gossypol. Chromatographs of cerebroside and phytoglycolipid acid hydrolysates show the presence of a long chain component that is thought to be a phytosphingosine.

### Phospholipids in Maturing Cottonseed

Changes in phospholipid composition during maturation of cottonseed are presented in Table VIII (34). The total phospholipid composition of five- and ten-day old cottonseed was very high (30.5% and 23.7% of oil, respectively). Between 15 and 40 days, the composition dropped to between 5.71% and 4.06%, and at 50 and 60 days, 3.20% and 2.38%. During the growing interval, lecithin increased from 25.8% at five days to 38.2% of total phospholipids at 60 days. Combined cephalin (phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine) percentages were higher than those of lecithin up to 50 days, then lessened as the seed matured to 60 days. Overall, the

individual phospholipids in the cephalin fraction vary during the maturing interval; only phosphatidylethanolamine decreases to a lower amount at 60 days compared to 5 days. Variations also were noted for phosphatidylcholine.

Except for the higher total saturated fatty acid content, most fatty acid amounts of the phospholipid fraction in the maturing cottonseed are similar to those in the oil from which they were separated (Table IX). The percentage of total saturated fatty acids, especially palmitic acid which makes up most of these fatty acids, decreases from 68.4% to 24.7% during the 60-day growing period; palmitic acid decreases from 59.0% to 23.1%. The unsaturated fatty acids, oleic and linoleic acids, increase significantly, 15.1% to 28.2% and 14.7% to 45.8%, respectively.

### **Gossypol-Phospholipid Reactions**

The heat and moisture of the old hydraulic press method of extracting oil from gossypol-containing glanded cottonseed caused most of the pigment to bind to constituents in the meal rather than those in the oil. Changes in oil extraction processes produced oils that contained considerable amounts of free gossypol pigments that bound to crude phospholipids and caused color and toxicity problems (5, 35). Cottonseed products, or blends containing gossypol, intended for human use in the United States must contain no more than 0.045% free gossypol (Food and Drug Administration). The Protein Advisory Group of the United Nations has set limits of 0.6% free gossypol and 1.2% total gossypol for human consumption in its programs (36). The dark-brown color caused by gossypol in cottonseed meal, oil and phospholipid extracts also limits their use in foods.

In general, cottonseed processing involves the following steps: (a) eliminate leaves, twigs, pieces of bolls and sand; (b) remove linters; (c) dehull; (d) screw press, prepress-solvent or solvent extract; and (e) desolventize, toast, and grind or pelletize products (Fig. 5; 37-39). The crude oil is purified by heating and treating with sodium hydroxide to remove soapstock, or foots, which contains most of the phospholipids. It is this refining process that removes the darker coloring materials such as gossypol by binding them to phospholipids to leave a clear yellow-colored oil. Bleaching clay is used to remove any remaining colored substances in the oil.

The ultraviolet spectrum ( $391 \mu$ ) of the total phospholipid extract and fractionation studies show that gossypol in cottonseed oil is present in a bound form with phosphatidylethanolamine and phosphatidylserine (Table X, 40). These authors also presented the IR spectra of the two phospholipids and showed that both of them had a band at  $6.2 \mu$  which is typical of a C=N

TABLE 4-VIII  
Phospholipid Composition (%) During Cottonseed Development and Maturity (34)

Phospholipid	5		10		15		20		30		40		50		60	
	In	O <sup>a</sup>	In	O	In	O	In	O	In	O	In	O	In	O	In	O
PI <sup>c</sup>	4.19	13.7	2.90	12.3	0.64	11.2	0.56	11.5	0.57	13.3	0.56	13.8	0.44	13.8	0.33	13.9
LS	4.96	16.3	3.62	15.3	0.75	13.1	0.61	12.5	0.56	13.0	0.52	12.8	0.41	12.8	0.30	12.6
PS	4.47	14.7	3.04	12.9	0.76	13.3	0.60	12.3	0.54	12.6	0.52	12.8	0.40	12.5	0.28	11.8
LE	7.87	25.8	7.19	30.4	1.73	30.3	1.58	32.4	1.40	32.6	1.31	32.3	1.09	34.1	0.91	38.2
PE	5.25	17.2	3.57	15.1	1.02	17.9	0.78	16.0	0.68	15.8	0.61	15.0	0.40	12.5	0.26	10.9
U	3.76	12.3	3.33	14.1	0.81	14.2	0.74	15.2	0.55	12.8	0.54	13.3	0.46	14.4	0.30	12.6
Total %	30.50		23.65		5.71		4.87		4.30		4.06		3.20		2.38	

<sup>a</sup>O = oil.

bp = total phospholipids.

cpi: phosphatidylinositol; LS: lysolecithin; PS: phosphatidylserine; LE: lecithin; PE: phosphatidylethanolamine; U: unknown phospholipid.

**TABLE 4-IX**  
**Fatty Acid Composition (%) of Mixed Phospholipid**  
**during Development and Maturity of Cottonseed (34)**

Fatty acid <sup>a</sup>	Days after flowering			
	5	15	30	60
C <sub>12:0</sub>	2.4	1.2	0.5	—
C <sub>14:0</sub>	5.9	1.8	0.9	0.6
C <sub>16:0</sub>	59.0	37.2	36.4	23.1
C <sub>16:1</sub>	1.6	0.6	1.5	1.3
C <sub>18:0</sub>	1.1	2.2	1.0	1.0
C <sub>18:1</sub>	15.1	22.2	23.6	28.2
C <sub>18:2</sub>	14.7	34.6	36.0	45.8

<sup>a</sup>Relative areas under the curves for the methyl esters of the component fatty acids as estimated from GLC tracings.

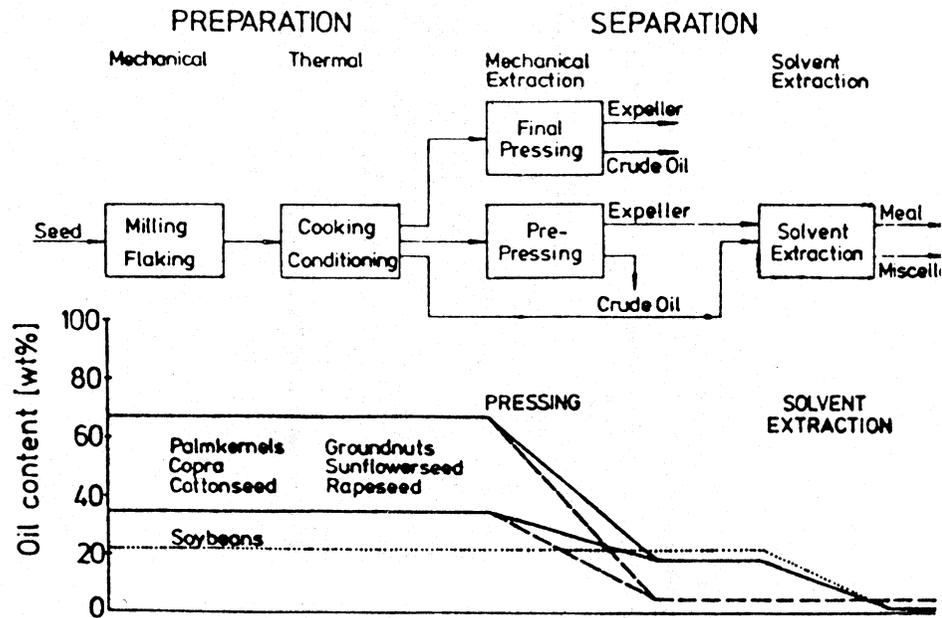


Fig. 4-5. Oil recovery processes. (38)

bond reactive with gossypol, i.e., interaction of the free amino group of the phospholipids with the aldehydes of gossypol. The components formed include mono- and diphosphatidylethanolamine-monogossypol, mono- and

diphosphatidylserine-monogossypol, and monophosphatidylethanolamine monophosphatidylserine-monogossypol (Fig. 6). Phosphorus, nitrogen, and gossypol IR spectra analyses revealed that N:P:gossypol ratios are 2.14:1.94:1 to 2.32:2.11:1 for phosphatidylethanolamine and 2.19:1.89:1 to 2.28:1.88:1 for phosphatidylserine (Table X; 22).

**TABLE 4-X**  
**Gossypol Composition (%) of Cottonseed Phospholipids**  
**from Two *Gossypium* Species (40)**

	Nitrogen (N)	Phosphorus (P)	Gossypol		Ratio of N:P: Gossypol
			Total	Free	
Phosphatidylethanolamine					
<i>G. barbadense</i>	1.47	2.93	25.64	0.06	2.14:1.94:1
<i>G. hirsutum</i>	1.21	2.41	19.21	0.04	2.32:2.11:1
Phosphatidylserine					
<i>G. barbadense</i>	1.27	2.31	20.95	0.04	2.28:1.88:1
<i>G. hirsutum</i>	1.10	2.11	18.85	0.03	2.19:1.89:1

### Glandless Cottonseed Phospholipids

Glandless or gossypol-free cottonseed now provides potential sources of commercial food-grade oil, lecithin or phospholipids, and meals or flours that are free of gossypol pigments, color and toxicity problems (5, 37–39).

Glandless cottonseed can be processed much more efficiently than those containing gland-filled gossypol to high quality edible kernel, oil and vegetable protein products (Fig. 7; 37–39). Kernels and meat fines can be flaked and extracted with hexane to produce high quality oil and defatted meal. With glandless cottonseed, there is no need for expensive heat treatments during processing to bind gossypol in the phospholipid fraction and meal. A refined oil from glandless cottonseed requires less purification processing, and as a result, less loss of neutral oil. The advent of glandless, or gossypol-free, cottonseed provides an opportunity to produce a food-grade phospholipid fraction as a by-product of edible oil products. Glandless cottonseed oil and lecithin products thus become more economically attractive by increasing revenues, decreasing waste-disposal costs and reducing emulsion problems that occur during processing.

The phospholipid and fatty acid compositions of the lecithin fraction

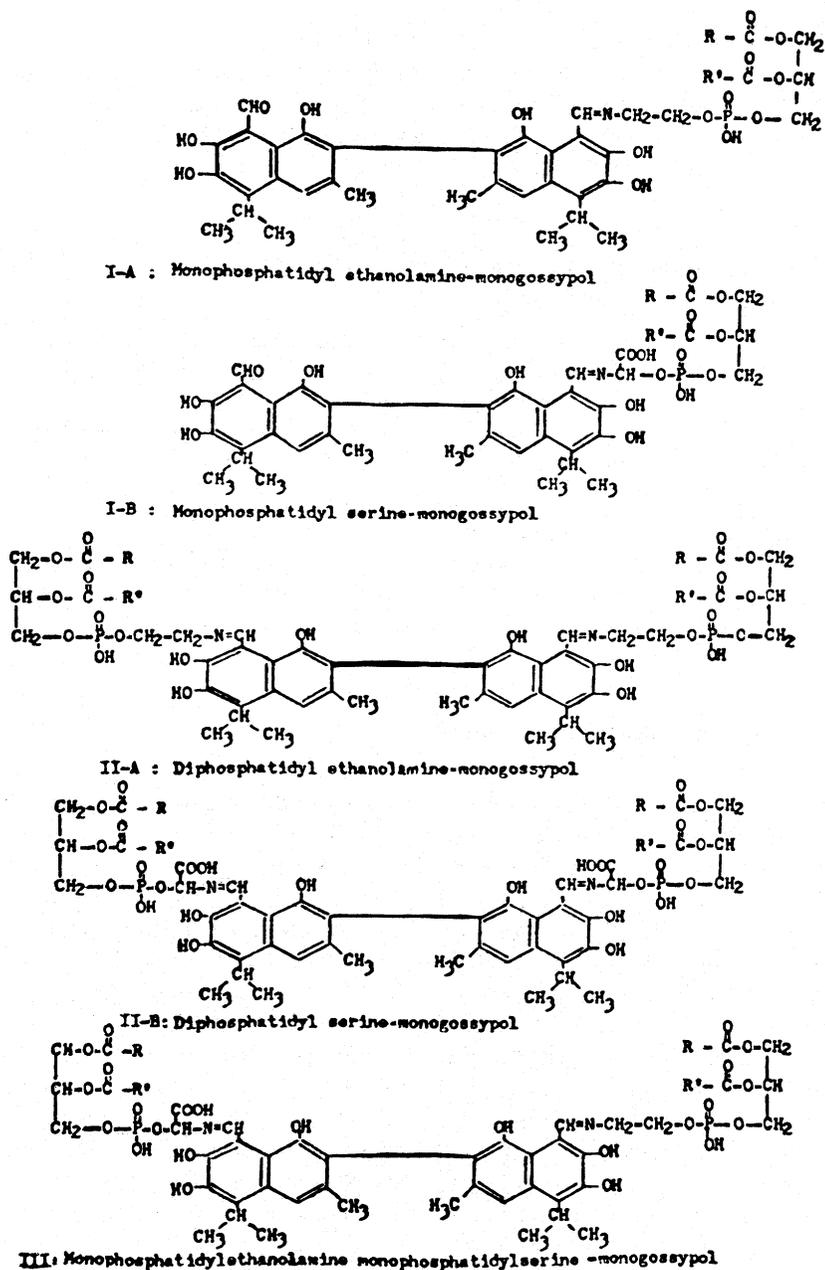


Fig. 4-6. Possible structures of cephalin-gossypol interactions. (40)

*Cottonseed Lecithin*

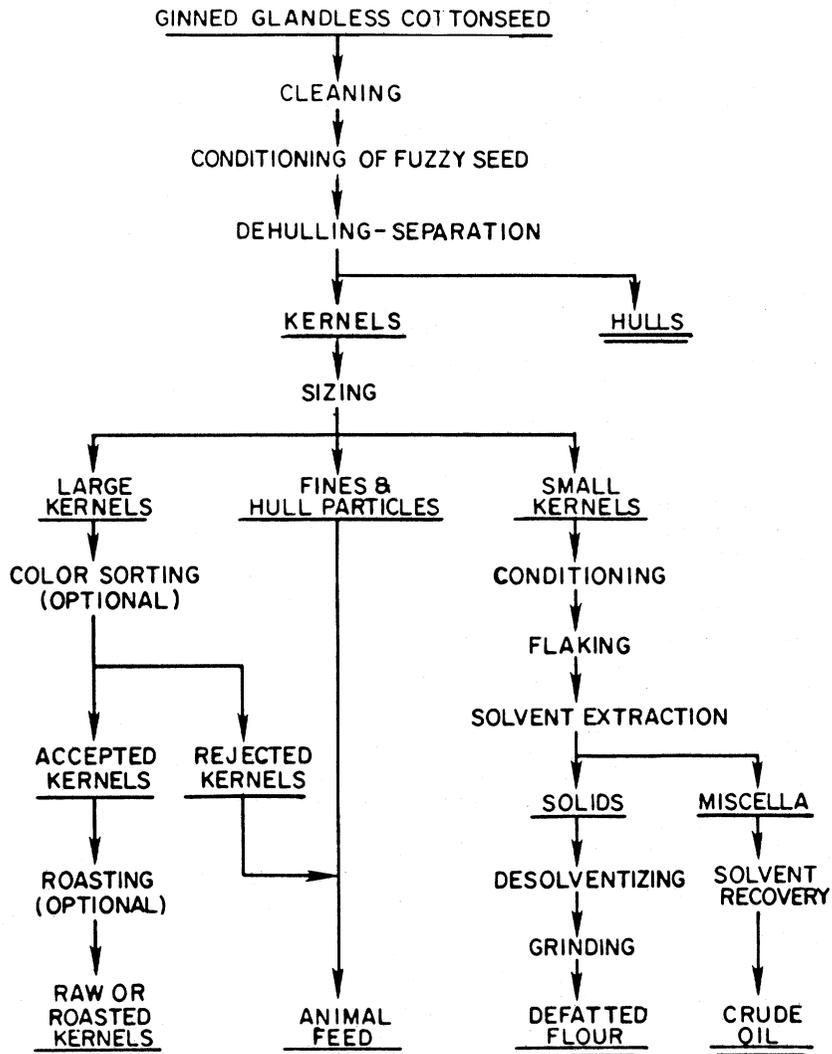


Fig. 4-7. Flow chart for production of glandless cottonseed kernels, flour, and oil. Solvent = hexane. (38)

from hexane-defatted glandless cottonseed oil are summarized in Tables XI and XII. Cottonseed phospholipids are superior to those of other oilseeds, especially soybeans, which presently are the main source of commercial lecithin (5). Since cottonseed oil contains only trace amounts of fatty acids

with more than two double bonds (linolenic acid), it is more stable to oxidation and rancidity processes, first indicated in earlier studies by Olcott (14). Other sources of phospholipids, e.g., soybeans, contain linolenic acid in amounts that can cause flavor, color and odor problems. Blending phospholipids from glandless cottonseed with those from other sources improves their flavor, color and odor properties during storage and use in food processing. Cottonseed phospholipids also contain high percentages of lecithin components that improve their functionality as food emulsifiers.

**TABLE 4-XI**  
**Composition of Phospholipid Fraction from Glandless Cottonseed Oil (37, 38)**

Phospholipid <sup>a</sup>	Composition (% of total phosphorus)
Origin	4.12
Lysophosphatidylcholine	2.56
Phosphatidylinositol	13.41
Phosphatidylserine	2.38
Phosphatidic acid	8.76
Phosphatidylcholine	23.16
Phosphatidylethanolamine	13.46
Phosphatidylglycerol	7.62
Lysophosphatidylserine	ND <sup>b</sup>
Lysophosphatidylethanolamine	ND
Unknown (sum: 6 TLC spots)	25.30

<sup>a</sup>Water (2–4%) was added to hexane-extracted glandless cottonseed oil, the resulting mixture stirred 30 min at 70°C, and centrifuged to separate the oil and phospholipid-containing fraction (41). The phospholipids were separated by 2-dimensional thin layer chromatography (TLC) on Silica gel-60 plates. Dimension I = chloroform: methanol:7N NH<sub>4</sub>OH (65:30:1); Dimension II = chloroform:methanol:acetic acid:water (170:25:25:4). Quantitation of the phospholipids was according to El-Sebajy et al. (42).

<sup>b</sup>ND = not detected.

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**TABLE 4-XII**  
**Fatty Acid Composition of Phospholipid Fraction**  
**from Glandless Cottonseed Oil (37, 38)**

Fatty acid	Composition (% of total fatty acids)
Myristic, C <sub>14:0</sub>	0.72
Palmitic, C <sub>16:0</sub>	24.19
Palmitoleic, C <sub>16:1</sub>	0.72
Stearic, C <sub>18:0</sub>	2.96
Oleic, C <sub>18:1</sub>	16.94
Linoleic, C <sub>18:2</sub>	53.58
Linolenic, C <sub>18:3</sub>	0.23
Arachidic, C <sub>20:0</sub>	0.41
Gadoleic, C <sub>20:1</sub>	0.08
Lignoceric, C <sub>24:0</sub>	0.18
Percentage of fatty acids recovered	49.90

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