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A Review of Lecithin Chemistry and Glandless Cottonseed as a Potential Commercial Source

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ABSTRACT

Industrial lecithin can be fractionated as phospholipids and glycolipids after neutral lipids and protein-containing contaminants are removed. The polar lipids are very reactive and are difficult to extract and purify from oilseeds. Their purity and special properties can be improved by a number of methods including solvent fractionation, hydrogenation, sulfonation, and ethoxylation. Studies are determining the role of the polar lipids of lecithin in (a) the synthesis of triglycerides in maturing seeds, (b) the structure of biological membranes, and (c) the molecular basis of the functionality of food ingredients. Lecithin, having both polar and nonpolar 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, prevents flavor deterioration of soybean oil and can be used to stabilize sunflower oil against color change during high temperature use. Gossypol binds to lecithin in oil from glanded cottonseed economically negating it as a commercial source of this product. New cultivars producing glandless, or gossypol-free cottonseed, may have potential as commercial sources of edible lecithin.

INTRODUCTION

At present, the only vegetable oil lecithin or crude phospholipid available for commercial use is a byproduct of processing soybean oil (1-3). In the past, phospholipids were extracted from animal materials such as egg yolk, brain tissue or spinal cord (1). These sources provided only small quantities of phospholipids, most of which were used pharmaceutically. Corn lecithin was reported to have certain superior properties for food use and was also available commercially (4). Before the early 1940s, cottonseed lecithin was commercially available and had properties superior to those of soybean lecithin (5-7), but changes in oil extraction processes produced oils that contained considerable amounts of free gossypol pigments which bound to crude phospholipids and caused color and toxicity problems (8,9). Glandless cottonseed now provides a potential source of commercial oil and lecithin that is free of gossypol pigments and color and toxicity problems (9).

"Lecithin" is the commercial or popular name for a naturally occurring mixture of several phospholipids including lecithin (phosphatidylcholine) and cephalin (phosphatidylethanolamine), phosphatidylinositol and phosphatidylserine. Brian (2) gives the proximate composition of commercial crude soybean lecithin as: phosphatidylcholine, 20%; phosphatidylethanolamine, 20%; phosphatidylinositol, 20%; soybean oil, 35%; and sugars, sterols and moisture, 5%. Commercial lecithin can be further processed to reduce oil content and bleached to improve color.

CLASSIFICATION

The most commonly used classification of lipids is an adaptation of Bloor's method by Deuel (10), in which lipids are divided into 3 groups: (a) simple lipids, which include neutral fats and waxes; (b) compound or conjugated lipids, which include phospholipids (Figs. 1 and 2), cerebrosides and sulfolipids; and (c) derived lipids, which include fatty acids, alcohols, hydrocarbons, and vitamins D, E and K. Folch (11) found that the phospholipid fraction referred to as "cephalin" consisted not only of phosphatidylethanolamine but could be separated into phosphatidylserine and one or more phosphatidylinositol compounds.

Lishkevich (12) found lecithin, or phospholipid, content of various oilseeds highest in cottonseed, followed by soybean, sunflower, flax, castor bean, and peanut seeds. He (13) also separated cottonseed phosphatides into 3 fractions: 16.5% was acetone soluble, of which 46.2% was lecithin, and 53.8% was cephalin; 76.5% was acetone insoluble, of which 53.2-59.4% was lecithin and 40.6-46.8% was cephalin; and 7.0% was benzene soluble which was almost entirely lecithin.

Most, if not all, phospholipids can exist in α- and β-forms. Daubert (14) illustrated α- and β-phosphatidylcholine, of which the α-form is optically active and the β-form is optically inactive. The β-phosphatidylcholine is not soluble in ethanol and is not extracted by the commonly used solvent systems. Daubert also illustrated the α- and β-forms of phosphatidylethanolamine and phosphatidylserine, but made no mention of phosphatidylinositol, nor of their optical activity. Tattrie (15) illustrated the α-acyl and β-acyl lysolecithins formed by the action of snake venom lecithinase A on phosphatidylcholine.

FUNCTIONS OF PHOSPHOLIPIDS

Seeds

High percentages of total phospholipids were noted in oil of cottonseed (Table I) harvested shortly (5 days) after flowering. These percentages decreased rapidly to a value of 2.4% in mature cottonseed harvested 60 days after flowering. Although the rates varied, each of the components of the total phospholipid fraction (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine) declined during cottonseed maturation.

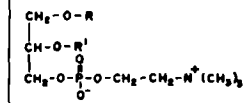
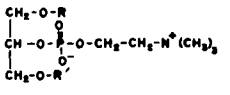
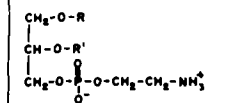
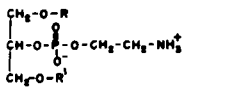
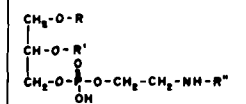
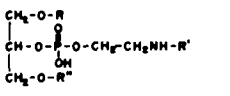
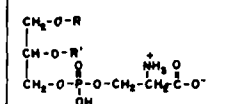
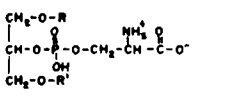
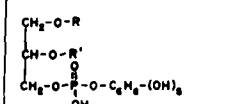
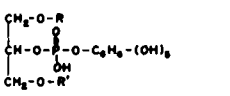
PHOSPHOLIPID	STRUCTURE: α-FORM	β-FORM	TRIVIAL NAME
PHOSPHATIDYL CHOLINE			LECITHIN
PHOSPHATIDYL ETHANOLAMINE			CEPHALIN
N-ACYL PHOSPHATIDYL ETHANOLAMINE			N.A.
PHOSPHATIDYL SERINE			CEPHALIN
PHOSPHATIDYL INOSITOL			CEPHALIN

FIG. 1. Structures of phospholipids. R,R′ = various fatty acids; cephalin (kephalin) currently refers to phosphatidylethanolamine; N.A. = nonavailable.

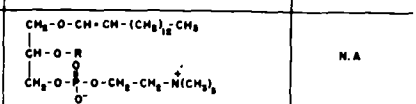
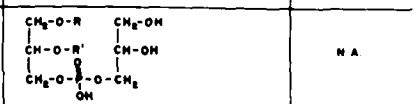
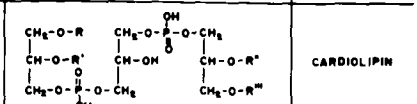
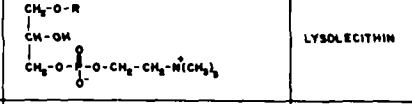
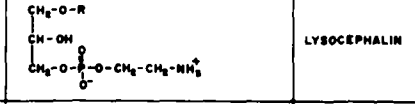
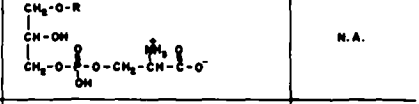
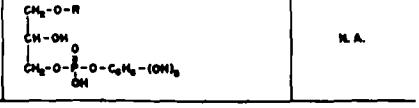
PHOSPHOLIPID	STRUCTURE: α-FORM	TRIVIAL NAME
SPHINGOMYELIN		N.A.
PHOSPHATIDYL GLYCEROL		N.A.
DIPHOSPHATIDYL GLYCEROL		CARDIOLIPIN
PHOSPHATIDIC ACID		N.A.
LYSOPHOSPHATIDYL CHOLINE		LYSOLECITHIN
LYSOPHOSPHATIDYL ETHANOLAMINE		LYSOCEPHALIN
LYSOPHOSPHATIDYL SERINE		N.A.
LYSOPHOSPHATIDYL INOSITOL		N.A.

FIG. 2. Structures of phospholipids with no available β-forms. R,R′ = various fatty acids, cephalin (kephalin) currently refers to phosphatidylethanolamine; N.A. = nonavailable.

Phospholipids also decreased during ripening of corn endosperm (Table II), but showed little change in maturing soybeans (Table I). As the cottonseed matured, the percentage of phosphatidylcholine increased; that of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol varied; that of saturated fatty acids decreased continually; and that of unsaturated fatty acids, oleic and especially linoleic, increased (Table III).

Fatty acid compositions of phosphatidylcholine extracts from seeds of cotton, peanut, sesame, mustard, barley and safflower show a range of about 16% saturated fatty acids for mustard seed to 36% for cottonseed (Table III). Safflower phosphatidylethanolamine and phosphatidylmyoinositol have higher levels of saturated fatty acids than phosphatidylcholine, and phosphatidylmyoinositol contains the most–36%. Myoinositol is one of 9 isomeric forms of inositol (myo- or meso-inositol, *cis* 1,2,3,5-*trans* 4,6-hexahydroxycyclohexane) and has vitamin activity.

The α and β fatty acid distribution in some seed phospholipids is given in Table IV. In the 5 seed sources shown, saturated fatty acids are found primarily in the α-position of phosphatidylcholine which is essentially what is found in the triglycerides (where the saturated fatty acids are present primarily in the α- and α′-positions and the unsaturated fatty acids in the β-position). Fatty acid distribution in the phosphatidylethanolamine and phosphatidylglycerol of gingko nuts is reversed, with the higher concentration of saturated fatty acids found in the β-position. Also, gingko nut phosphatidylethanolamine and phosphatidylglycerol had higher concentrations of total saturated fatty acids than did phosphatidylcholine of any of the 5 seed sources shown.

Dawson et al. (32) isolated and characterized a phospholipid, N-acyl phosphatidylethanolamine (APE), that constitutes ca. 5% of the phospholipids in peas at the start of germination but decreases markedly during the first 24 hr thereafter. An even lower initial concentration of APE was

TABLE I

Phospholipid Content of Maturing Seeds (%)

Phospholipid	Days after flowering					
	Cottonseed (22)			Soybean (23)		
	5	30	60	9	55	97
Phosphatidylcholine	25.8	32.6	38.2	2.0	3.6	45.0
Phosphatidylethanolamine	17.2	15.8	10.9	3.5	1.4	26.3
Phosphatidylserine	14.7	12.6	11.8	–	–	–
Phosphatidylinositol	13.7	13.3	13.9	6.3	15.3	14.1
Phosphatidylglycerol + diphosphatidylglycerol	–	–	–	3.3	2.7	3.3
Phosphatidic acid	–	–	–	64.2	51.8	5.0
Lysophosphatidylcholine	16.3	13.0	12.6	–	–	–
Unknown	12.3	12.8	12.6	20.6	25.4	6.3
% in oil	30.5	4.3	2.4	51.5	8.1	10.2

TABLE II

Phospholipid Content of Developing Maize Kernels (%) (24)

Phospholipid	Days after pollination								
	9			42			87		
	Germ	Endosperm	Pericarp[a]	Germ	Endosperm	Pericarp	Germ	Endosperm	Pericarp
Phosphatidylcholine	46.7	47.2	51.2	55.9	15.2	40.7	57.0	7.2	22.2
Phosphatidylethanolamine[b]	33.3	28.8	16.7	20.4	8.3	16.0	17.0	3.1	11.1
Phosphatidylinositol	16.7	16.7	11.9	18.2	8.0	12.3	21.6	4.6	22.2
Lysophosphatidylcholine[c]	trace	1.4	8.3	1.8	53.6	3.7	1.4	68.8	11.1
Lysophosphatidylethanolamine	3.3	3.1	3.6	1.7	7.4	6.2	0.6	12.5	trace
Phosphatidic acid	trace	2.8	8.3	2.0	7.4	21.0	2.3	3.8	33.2
Total[d]	30	354	84	599	841	82	647	584	9

[a]Sixteen days after pollination.

[b]Includes some phosphatidylglycerol.

[c]Includes some lysophosphatidylglycerol.

[d]μg phospholipid/seed part.

found in spring beans and soybeans and this, too, decreased after hydration for 48 hr. Oat seeds, however, have an initial APE concentration of ca. 12% of the phospholipids, and retain most of it after hydration for 60 hr with no equivalent loss of total phospholipid phosphorus during this germination period. Low concentrations of APE were also found in turnips, carrots and radish seeds. Wheat flour (33) and a winter wheat cultivar (21) were also found to contain significant amounts of APE, which appears to be a widely occurring component of plant seeds. Dawson et al. (32) give the fatty acid composition of APE from peas and the O-acyl fatty acids (which are alkali-labile) and the N-acyl fatty acids (which are alkali-stable); a much higher concentration of saturated fatty acids was found with the N-linkage than with the O-linkage in the APE.

Phospholipids are widely distributed in plant and animal cells and undergo breakdown and resynthesis to produce lipid precursors for triglyceride biosynthesis (34). Phospholipids of the same "class," differing only in fatty acid composition, may exhibit vastly different metabolic rates. The role of the widely distributed enzymes is critical in exchange reactions involving cleavage of fatty acids and reacylation, or cleavage of the base and re-esterification, to meet particular requirements of the living organism at various growth stages.

Phospholipids probably serve as carriers at the site where fatty acids are desaturated; studies show that when a radioactively labeled substrate is fed to leaf tissue, it appears initially in phosphatidylcholine (35). The label within phosphatidylcholine is transferred first to oleic acid, then to linoleic acid, and finally to linolenic acid. These results were interpreted as indicating that conversion of oleic acid to linoleic and linolenic acids took place within the phospholipid molecule—a conclusion that has been supported by the finding of an enzyme in the microsomal fraction of safflower seed that enhances reactions involving desaturation of oleic acid to linoleic acid in the presence of phosphatidylcholine.

Membranes

Phospholipids are essential components of cytoplasmic membranes of vegetative and reproductive tissues (36), and play an important role in germination and maturation of seeds. Phospholipids in cell membranes are involved in the movement of charged and uncharged molecules, transport of triglycerides, control of enzyme activities as well as the role in triglyceride biosynthesis.

The interaction between polar and nonpolar groups of lipid (including phospholipids) and protein is an important feature of models of membrane structure (36,37). In this model (Fig. 3), mushroom-shaped or dumbbell-shaped protein moieties are arranged with their narrower shafts or stalks penetrating into or through a lipid bilayer, and their more bulbous heads located outside the lipid to form predominantly nonlipid layers.

Foods

The model for the living cell membrane (Fig. 3) helps explain the interactions of proteins and phospholipids in such food systems as emulsions. Researchers (38,39) have reported that native soybean proteins do not interact

TABLE III

Fatty Acid Composition of Various Seed Phospholipids and Triglycerides (%)

	Cottonseed										
					Triglyceride			5 days after flowering[b]		60 days after flowering[b]	
Fatty acid[a]	Phospholipid (8)	Triglyceride (8)	Phospholipid (25)	Triglyceride (25)	Glandless cottonseed oil (26)	Glanded cottonseed oil (26)	Phosphatidylcholine (27)	Phospholipid (22)	Triglyceride (22)	Phospholipid (22)	Triglyceride (22)
12:0	–	–	–	–	–	–	–	2.4	4.2	–	–
14:0	0.3	0.3	–	1.4	0.7	0.9	–	5.9	2.3	0.6	0.7
16:0	27.5	21.9	17.3	23.4	22.6	23.0	32.1	59.0	35.9	23.1	23.4
16:1	–	–	1.5	2.1	–	–	–	1.6	3.6	1.3	1.2
18:0	–	–	7.3	1.1	2.1	2.2	2.4	1.1	1.0	1.0	1.2
18:1	20.2	11.4	20.3	22.9	17.7	17.7	25.5	13.1	26.9	28.2	26.8
18:2	57.1	66.4	44.4	47.8	56.5	55.8	40.0	14.7	26.0	45.8	46.7
18:3	–	–	–	–	–	–	–	–	–	–	–
20:0	–	–	2.8	1.3	–	–	–	–	–	–	–
20:1	–	–	–	–	–	–	–	–	–	–	–
20:2	–	–	–	–	–	–	–	–	–	–	–
20:3	–	–	–	–	–	–	–	–	–	–	–
20:1-20:5	–	–	–	–	–	–	–	–	–	–	–
22:0	–	–	–	–	–	–	–	–	–	–	–
22:1	–	–	–	–	–	–	–	–	–	–	–
24:0	–	–	–	–	–	–	–	–	–	–	–
20:0,22:1,24:0	–	–	–	–	–	–	–	–	–	–	–
20:0,22:0,24:0	–	–	–	–	–	–	–	–	–	–	–
C20+C22 unsat. as C26	–	–	6.4	–	–	–	–	–	–	–	–
Unknown	–	–	–	–	0.4	0.4	–	–	–	–	–
Saturated fatty acids	22.8	22.2	27.4	27.2	25.4	26.1	34.5	68.4	43.4	24.7	25.3

	Soybean				Peanut			Sunflower	
	Phospholipid (25)	Triglyceride (25)	Phospholipid (28)	Triglyceride (28)	Phospholipid (25)	Triglyceride (25)	Phosphatidylcholine (27)	Phospholipid (25)	Triglyceride (25)
12:0	–	–	–	–	–	–	–	–	–
14:0	–	0.3	–	–	–	–	–	–	–
16:0	11.7	9.8	15.8	16.4	16.2	8.3	23.5	14.7	5.6
16:1	8.6	0.5	–	–	–	–	–	–	–
18:0	4.0	2.4	6.3	3.9	2.8	3.1	6.6	5.1	2.2
18:1	9.8	22.9	13.0	29.2	47.1	56.0	54.8	19.3	25.1
18:2	55.0	50.7	63.0	53.6	22.7	26.0	15.1	45.9	66.2
18:3	5.5	6.5	2.0	2.3	–	–	–	–	–
20:0	1.4	0.9	–	0.6	–	–	–	9.5	0.9
20:1	–	–	–	–	–	–	–	–	–
20:2	–	–	–	–	–	–	–	–	–
20:3	–	–	–	–	–	–	–	–	–
20:1-20:5	–	–	–	–	–	–	–	–	–
22:0	–	–	–	–	–	–	–	–	–
22:1	–	–	–	–	–	–	–	–	–
24:0	–	–	–	–	–	–	–	–	–
20:0,22:1,24:0	–	–	–	–	–	–	–	–	–
20:0,22:0,24:0	–	–	–	–	4.6	6.6	–	–	–
C20+C22 unsat. as C26	5.5	–	–	–	4.1	–	–	5.5	–
Unknown	–	–	–	–	2.5	–	–	–	–
Saturated fatty acids	17.1	13.4	22.1	20.9	26.1	18.0	30.1	29.3	8.7

	Rapeseed						Barley		Oats		Linseed	
			Medium erucic acid[b]		Low erucic acid[b]							
	Phospholipid (25)	Triglyceride (25)	Phospholipid (29)	Triglyceride (29)	Phospholipid (29)	Triglyceride (29)	Phospholipid (30)	Triglyceride (30)	Phospholipid (18)	Triglyceride (18)	Phospholipid (26)	Triglyceride (26)
12:0	–	–	–	–	–	–	–	–	–	–	–	–
14:0	0.8	–	–	–	–	–	1.0	0.3	2.4	1.2	–	0.2
16:0	8.3	2.0	12.1	3.8	12.7	5.2	25.8	21.7	5.7	13.8	11.3	5.4
16:1	2.1	–	1.1	0.1	0.8	0.3	–	–	–	–	3.5	–
18:0	–	–	0.8	1.6	2.1	1.9	1.9	1.1	5.7	2.3	10.6	3.5
18:1	22.4	17.0	43.9	39.2	42.3	59.8	11.0	15.9	28.5	44.4	33.6	18.8
18:2	42.2	29.0	33.1	20.5	31.4	20.4	56.4	54.7	50.6	35.2	20.4	24.2
18:3	trace	–	5.9	9.2	8.3	10.8	4.0	6.2	3.8	2.0	17.4	47.3
20:0	–	–	–	–	–	0.4	–	–	–	–	–	0.6
20:1	–	–	1.9	11.7	1.2	0.9	–	–	–	–	–	–
20:2	–	–	–	–	–	–	–	–	–	–	–	–
20:3	–	–	–	–	–	–	–	–	–	–	–	–
20:1-20:5	–	–	–	–	–	–	–	–	2.9	0.6		
22:0	–	–	–	–	–	–	–	–	–	–	–	–
22:1	22.7	51.0	1.2	14.9	1.1	0.6	–	–	–	–	–	–
24:0	–	–	–	–	–	–	–	–	–	–	–	–
20:0,22:1,24:0	–	–	–	–	–	–	–	–	0.5	0.1		
20:0,22:0,24:0	1.5	1.0	–	–	–	–	–	–	–	–	–	–
C20+C22 unsat. as C26	–	–	–	–	–	–	–	–	–	–	–	0.6
Unknown	–	–	–	–	–	–	–	–	–	–	–	–
Saturated fatty acids	10.6	3.0	12.9	5.4	14.8	7.5	28.7	23.1	14.2	17.4	21.9	9.7

	Ginkgo nuts		Sesame	Mustard	Barley	Safflower		
	Phospholipid (19)	Triglyceride (19)	Phosphatidylcholine (27)	Phosphatidylcholine (27)	Phosphatidylcholine (20)	Phosphatidylcholine (31)	Phosphatidylethanolamine (31)	Phosphatidylinositol (31)
12:0	–	–	–	–	trace	–	–	–
14:0	0.4	trace	–	–	0.4	0.2	0.2	0.1
16:0	27.5	9.4	15.3	14.2	19.5	13.8	19.5	29.0
16:1	4.2	4.1	–	–	0.4	–	–	–
18:0	1.7	3.2	10.3	1.3	1.6	4.1	2.9	4.8
18:1	29.2	45.9	37.4	53.1	18.8	12.1	8.3	4.4
18:2	30.9	30.1	37.0	28.0	65.3	68.1	69.1	59.7
18:3	0.6	trace	–	3.4	4.0	0.1	–	–
20:0	–	–	–	–	–	0.4	–	0.7
20:1	0.6	1.5	–	–	–	–	–	–
20:2	3.0	2.3	–	–	–	–	–	–
20:3	–	3.3	–	–	–	–	–	–
20:1-20:5	–	–	–	–	–	–	–	–
22:0	1.9	–	–	–	–	0.5	–	0.7
22:1	–	–	–	–	–	0.4	–	–
24:0	trace	–	–	–	–	–	–	–
20:0,22:1,24:0	–	–	–	–	–	–	–	–
20:0,22:0,24:0	–	–	–	–	–	–	–	–
C20+C22 unsat. as C26	–	–	–	–	–	–	–	–
Unknown	–	–	–	–	–	0.3	–	0.6
Saturated fatty acids	31.5	12.6	25.6	15.5	21.6	19.3	22.6	35.9

[a]Fatty acid, indicated by carbon number and the number of double bonds.

[b]Expressed as fatty acid methyl esters.

TABLE IV

Distribution of α and β Fatty Acids in Some Seed Phospholipids (%)

Fatty acid[a]	Phosphatidylcholine								Phosphatidyl-ethanolamine		Phosphatidyl-glycerol	
	Cottonseed		Groundnut		Sesame		Mustard		Ginkgo nut		Ginkgo nut	
	α	β	α	β	α	β	α	β	α	β	α	β
	(27)		(27)		(27)		(27)		(19)		(19)	
14:0	—	—	—	—	—	—	—	—	1.0	3.1	1.8	8.0	1.3	0.9
15:0	—	—	—	—	—	—	—	—	0.3	trace	0.7	1.9	0.4	0.6
16:0	55.6	8.6	42.8	4.1	26.8	3.8	25.0	3.3	48.0	13.3	38.5	60.5	30.1	50.6
16:1	—	—	—	—	—	—	—	trace	3.9	trace	7.0	1.3	4.3	1.5
18:0	4.8	—	13.2	—	20.6	—	2.6	—	0.2	1.5	trace	1.0	0.5	1.2
18:1	21.4	29.5	37.6	72.0	28.6	46.1	53.8	52.3	77.8	40.2	4.5	5.5	7.1	11.5
18:2	18.0	61.9	6.2	23.9	23.8	50.1	16.6	39.4	10.2	14.4	34.3	14.9	36.8	31.6
18:3	—	—	—	—	—	—	1.8	5.0	0.3	0.3	—	2.7	3.4	trace
20:1	—	—	—	—	—	—	—	—	2.1	4.0	—	trace	trace	0.4
20:2	—	—	—	—	—	—	—	—	2.6	22.5	10.8	4.0	12.9	trace
22:0	—	—	—	—	—	—	—	—	4.1	0.3	3.6	trace	2.4	—
22:1	—	—	—	—	—	—	—	—	—	0.4	—	—	—	—
24:0	—	—	—	—	—	—	—	—	—	—	—	—	0.6	—
Saturated fatty acid	60.4	8.6	56.0	4.1	47.4	3.8	27.6	3.3	52.6	18.2	44.6	71.4	35.3	54.8

[a]Fatty acid, indicated by carbon number and the number of double bonds.

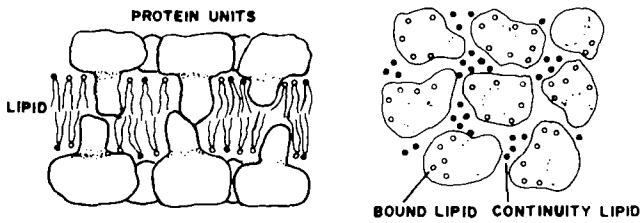


FIG. 3. Diagram of a cell membrane structure. Protein units penetrate into or through the central layer of lipid. Protein, in either compact or extended form, occupies only about one-half of the external layers; the remainder is occupied mainly by water. Source: Zahler and Weibel (37).

well with lecithin; but, after soybean proteins are dissociated into subunits or unfolded polypeptides, lipoproteins form from interactions with phospholipids. In model systems, attainment of equilibrium tension at the surface of a colloidal liquid by phospholipids and proteins is diffusion-dependent, being influenced by concentration and mobility of proteins and their surface charges, ease with which proteins unfold, and their facility for packing as lipoproteins at the interface. Proteins with high molecular flexibility (that unfold readily) show high activity at the surface of the colloidal liquid because facile unfolding exposes hydrophobic and hydrophilic regions that interact with phospholipids and enhance interfacial film formation.

EXTRACTION PROCEDURES

Standard research procedures for isolating the total phospholipid portion of plant material (Table V) include solvent extraction of the lipids and application of various separation and purification steps to the extract to fractionate and quantitate the individual phospholipids. Select methods are summarized as follows:

Folch et al. (47) General Method

The tissue is first extracted twice with a chloroform and methanol (2:1) mixture, then the 2 extracts are mixed with water or 0.1 M potassium chloride to form a 2-phase system whereby the gangliosides and nonlipid materials are separated from the phospholipids. This method extracts 92-97% of the total lipids.

Bligh and Dyer (51) General Method

The tissue is first extracted with a chloroform, methanol and water (1:2:0.8) monophasic system, after which the extract is mixed with water to form a 2-phase system. The aqueous phase contains the nonlipid materials and most of the gangliosides. A second extraction is run with a chloroform, methanol and water (2:2:1.8) biphasic system. The tissue residue in the biphasic extract assists in separating the nonlipid components from the phospholipids.

Rouser and Fleischer (53) Exhaustive Method

This method extracts all phospholipids except those that are covalently bonded and require enzymatic, acidic, or basic hydrolysis prior to their extraction. The procedure consists of 6 sequential steps using solvent mixtures of varying proportions of chloroform and methanol; aqueous 28% ammonium hydroxide is added to the fourth solvent mixture, glacial acetic acid and water to the fifth, and concentrated hydrochloric acid to the sixth. Most tissues do not require all 6 steps and an abbreviated sequence of the first 4 steps may be used. The extracts from the first 4 extractions are combined separately from those of the fifth and sixth extractions.

TABLE V

Phosphatide Extraction Methods for Plant and Animal Tissues

Material extracted	Reference	Comments
General method:		
Cocoa beans	J.G. Parsons and P.B. Price (20)	
Corn	E.J. Weber (40)	
Cottonseed	T.J. Jacks et al. (41)	Compares 6 methods
Food products (chocolate, milk powder, etc.)	A. Dieffenbacher and U. Bracco (42)	
Rapeseed	M.E. McKillican and J.A.G. Larose (43)	
	R. Zadernowski and F. Sosulski (29)	
Oats	M.R. Sahasrabudhe (18)	Compares 7 methods
Safflower	H.J. Burkhardt (31)	
Soybeans	S. Temple (44)	Compares several methods
	H.P. Kaufman and H.O. von Orde (45)	
	O.S. Privett et al. (23)	
Wheat	R.O. Daftary and Y. Pomeranz (46)	
	J.A. de la Roche et al. (21)	Compares 6 methods
Brain	J. Folch et al. (47)	
Egg yolk	B. Ramesh et al. (48)	
Herring	B. Brozdowski and R.G. Ackman (49)	
	G. Lunde et al. (50)	
Cod	E.G. Bligh and W.J. Dyer (51)	
Exhaustive method:		
Soybeans	A.V. Zhukov and A.G. Vershchagin (52)	Requires special apparatus
Animal organs (heart, liver, kidney, brain, mitochondria)	G. Rouser and S. Fleischer (53)	
Enzyme inactivation pretreatment:		
Ginkgo nuts	C. Urakami et al. (19)	
Plant leaves	M. Kates (54)	

Kates (54) Phospholipidase Control Method

This procedure provides a means of extracting lipids from plant tissues that contain very stable and active phospholipases that are capable of degrading up to 40% of the lipids within a few minutes of the start of aqueous chloroform and methanol extraction. The tissues are frozen in liquid nitrogen and powdered, and the powdered tissue is extracted and washed with boiling 2-propanol followed by extracting and washing with 2-propanol and chloroform (1:1), and then is finally washed with chloroform.

All of these procedures also include steps to concentrate the crude phospholipid extracts, remove the nonphospholipid impurities by solvent fractionation, and selectively precipitate the phospholipids. Further separation of the phospholipids into glycerol-phospholipid and inositol-phospholipid fractions can be achieved by selective extraction procedures. Solvent-solvent countercurrent fractionation concentrates and separates some phospholipids. Many phospholipids complex with metal salts, permitting them to be isolated or removed from a mixture. The cadmium chloride complex with lecithin, e.g., can be isolated from a mixture of phospholipids because of its solubility in ether. Chromatographic column separation procedures using such absorbents as aluminum oxide, aluminum silicate, or magnesium oxide will separate some phospholipids or classes of phospholipids with similar groups. Fractional crystallization can also be used to separate and purify certain phospholipids and thin layer chromatographic procedures are useful for separating, identifying, and quantifying small samples of phospholipids as well as for monitoring other separation procedures.

Commercial Method

Commercial phospholipids (lecithin) are byproducts of industrial processing of oilseeds (Fig. 4). After dehulling, flaking, and cooking oilseeds, the oil is removed by one of 4 basic methods: hydraulic pressing, screw pressing, prepress solvent extraction, or direct solvent extraction.

Prior to degumming, the crude oil is filtered to remove meal fines, after which the oil is hydrated by thoroughly mixing a controlled amount of water (2-3%) with the oil at 50-70 C. The α-form phospholipids are the principal components of the sludge, which also contains other lipid and nonlipid materials. The sludge formed during hydration (which contains 40-50% water) is removed from the oil by centrifugation and the crude phospholipid sludge is dried to a moisture content of less than 1% (to provide good storage stability and fluidity). Precise control of temperature and residence time during processing is required to obtain a good quality, light-colored product that can be stored at 20-30 C for months without significant change in quality. The β-form phospholipids and the calcium and magnesium salts of phosphatidic and lysophosphatidic acids are nonhydratable and are removed from the degummed oil by caustic refining.

Further purification of crude phospholipids is required when their use requires a neutral flavor, light color, or absence of oil (crude phospholipids contain 30-40% unrefined oil). Extraction with acetone removes the oil, and to some extent, the pigments. Further improvement in

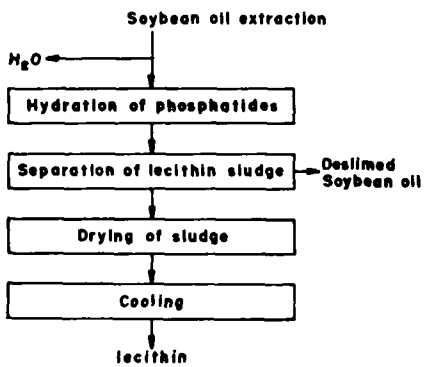


FIG. 4. Processing steps in the production of crude phospholipids (lecithin) from soybean oil. Source: Van Niewenhuyzen (3).

color can be obtained by a simple bleach with hydrogen peroxide or a double bleach by also adding benzoyl peroxide. The purified phospholipids can be dissolved in refined oil or processed into a powdered or granulated form.

Modification of the phospholipids by various means can change their physicochemical characteristics, and, in turn, their emulsifying, stabilizing and dispersing properties (Fig. 5). Fractionation of the phospholipids in 90% ethanol changes the ratio of phosphatidylcholine and phosphatidylethanolamine to greater than 5 to 1 and produces a product with improved emulsifying and antispattering properties for use in saltless margarines. Partial hydrolysis of the phospholipids by phospholipase A, acids, or alkali produces a product with improved hydrophilic and emulsifying properties, and reduced calcium sensitivity which makes it useful as a milk replacer. Enzyme hydrolysis specifically removes fatty acids at the β-position, providing better control of the final product and its properties than acid or base hydrolysis. Acetylation (in which acetic anhydride acetylates the amino group of the phosphatidylethanolamine) improves emulsifying properties and permits fractionation of phosphatidylcholine which also improves emulsification. Acetylation can be performed during degumming, with the sludge, or with dried crude phospholipids. Hydroxylation improves the emulsifying properties of crude phospholipids, and their dispersibility in cold water. Hydroxyl groups are formed in unsaturated fatty acid groups in the presence of high concentrations of hydrogen peroxide and lactic acid.

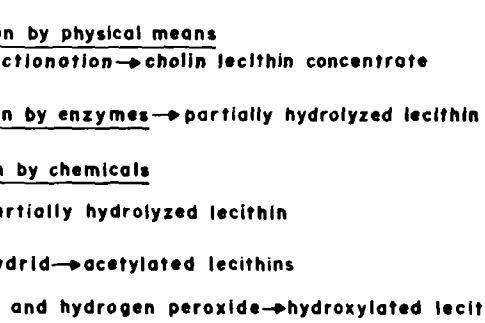


FIG. 5. Production of specialized lecithin products by physical and chemical modification. Source: Van Nieuwenhuyzen (3).

USES

Phospholipids are used as emulsifiers and antioxidants in such food and drug products as candies, margarines, shortenings, chocolates, baked goods, ice cream, ointments, salves, creams, and in vehicles for dispersing drugs, vitamin, liver extracts and cosmetics (Table VI). They are also used as emulsification or surface-active agents and antioxidants in petroleum, leather, paint, surface coating and rubber products. These uses generally take advantage of the primary property of the phospholipids, which is to lower the surface tension of the materials they are incorporated into, and to make homogeneous systems out of mixtures of materials or compounds and immiscible phases.

COTTONSEED PHOSPHOLIPIDS

Among common oilseeds, cottonseed has the highest content of phospholipids other than soybean. Cottonseed phospholipids contain less phosphatidylcholine, but more phosphatidylethanolamine and phosphatidylinositol than peanut and soybean seeds (Table VII).

Gas liquid chromatographic analysis of fatty acid methyl esters showed that total lecithin of the major oilseeds (cottonseed, soybean and peanut) contains 60-80% unsaturated fatty acids (Table III). The fatty acids in the β-position of cottonseed lecithin that are liberated by phospholipase-A hydrolysis consist mainly of oleic (29.5%) and linoleic (61.9%) acids. Cottonseed lecithin also contains 8.6% palmitic acid in this position. Estimates of lecithin classes in terms of saturated and unsaturated fatty acids in the α- and β-positions show that among the major oilseeds, the amount of disaturated lecithin is negligible and the content of α-unsaturated, β-saturated types are very low (Table IV). Cottonseed and peanut seed lecithins contain similar amounts (60.3% and 56.0%, respectively) of α-saturated, β-unsaturated fatty acids; soybean contains approximately one-half that amount of these fatty acids. The diunsaturated lecithin content of soybean was twice that of cottonseed and peanut seed. Egg lecithin fatty acid, by comparison, is mostly the α-saturated, β-unsaturated type.

There are few differences in the percentage of phospholipids in cottonseed oil prepared by different extraction methods normally used in the oil crushing industry (Table VII). Similarly, the percentage of individual components in the phospholipid fraction of cottonseed oil prepared by these typical extraction techniques does not vary greatly.

Composition of the acetone precipitate, the major phospholipid fraction of cottonseed oil, is summarized in Table VIII. Overall, cottonseed oil contains about 2.2% phospholipids. This residue consists of 53% fatty acids, most of which are palmitic, oleic and linoleic acids. Lecithin makes up about 35% of the phospholipids in cottonseed oil, and this component contains high amounts of oleic, palmitic and linoleic acids. The combined amount of phosphatidylethanolamine and phosphatidylserine in the phospholipid fraction is about equal to the amount of lecithin. Palmitic, oleic and linoleic acids make up most of the fatty acids. Compared to lecithin, phosphatidylethanolamine and phosphatidylserine contain much higher percentages of total gossypol, slightly more palmitic acid, and less linoleic acid.

The presence of gossypol in the phospholipid extracts of cottonseed negates their use in foods; there is extensive evidence of gossypol's toxicity to different animal species (55). Cottonseed products, or blends containing these substances, that are 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 their programs. The dark-brown color caused by gossypol in cottonseed phospholipid extracts also limits their use in foods.

The ultraviolet spectrum of the total phospholipid extract (391 μ), and fractionation studies presented in Table VIII show that gossypol in cottonseed oil is present in a bound form with phosphatidylethanolamine and phosphatidylserine (8); the components formed include monophosphatidylethanolamine–monogossypol, and diphosphatidylethanolamine–monogossypol.

GLANDLESS COTTONSEED– A SOURCE OF PHOSPHOLIPIDS

Cottonseed phospholipids were marketed only to a small extent in the past. The heat and moisture of the old hydraulic press method of extracting oil from cottonseed caused gossypol to bind constituents of the meal. New methods (the screw press method, and, more recently, the prepress-solvent and solvent extraction methods) also extract gossypol, which, in turn, binds to the phospholipids.

But the advent of glandless (or gossypol-free) cottonseed

TABLE VI

Phospholipid Uses and Functions in Various Products

Product	Function	Effect
Food		
1. Baked goods	Gluten chemistry modifier; emulsifier and stabilizer for fats; antioxidant; wetting agent.	Improves "shortening effect," emulsification, flavor, shelf-life, texture, and moisture retention.
2. Pasta products Dried potatoes	Inclusion partner for amylose; co-emulsifier for mono- and diglycerides; antioxidant.	Improves emulsification, flavor, texture and shelf-life.
3. Wafers	Emulsifier; antioxidant.	Improves emulsification, flavor, texture and shelf-life.
4. Cream-like emulsions	Emulsifier; stabilizer; antioxidant.	Improves emulsification and stabilizes emulsion; improves flavor, texture and shelf-life.
5. Candy	Emulsifier; viscosity reducer; wetting agent; dispersant.	Aids mixture of sugar, fats and water to prevent greasiness, graining and streaking.
6. Margarine Shortening	Emulsifier; antioxidant.	Decreases spattering; improves emulsification, flavor, texture and shelf-life.
7. Meat (sliced bacon)	Releasing agent; antioxidant.	Improves separation of refrigerated slices and shelf-life.
8. Instant foods Whole milk powder	Instantizer; emulsifier; antioxidant; dispersing agent; wetting agent; nutritional supplement.	Speeds and improves reconstitution, texture, emulsification and shelf-life.
9. Dietetic foods	Emulsifier; antioxidant; wetting and strengthening agent; nutritional supplement.	Improves emulsification, flavor, texture and shelf-life.
10. Pan coatings	Release and lubricate.	Improves appearance and shelf-life; prevents surface greasiness.
Pharmaceuticals	Emulsifier; carrier; antioxidant; donates choline and linoleic acid.	Improves emulsification, dispersion and shelf-life.
Cosmetics	Emulsifier and foam stabilizer; emollient; dispersing agent; resorbable refatting agent with depth effects; wetting agent; antioxidant; donates choline, inositol, and linoleic acid. Vitamin source: pantothenic acid, thiamine, folic acid, riboflavin, pyridoxine, biotin and niacin.	Improves dispersion of various components (pigments) and shelf-life.
Feedstuffs		
1. Dog food	Emulsifier; wetting agent; antioxidant; nutritional supplement; releasing agent.	Aids blending of fats and water, and clean release from equipment and can; improves animal coat glossiness.
2. Calf and sow milk	Emulsifier; wetting agent; antioxidant; nutritional supplement.	Improves feed utilization and nutrition.
3. Poultry feed	Emulsifier; wetting agent; antioxidant; nutritional supplement, releasing agent.	Improves emulsification and dispersion, feed utilization and nutrition; prevents lodging of food in poultry beak and resulting necrosis.
4. Livestock feed	Emulsifier; wetting agent; antioxidant; nutritional supplement; releasing agent; anti-dusting agent.	Improves emulsification and dispersion, food utilization, and nutrition.
Industrial		
1. Insecticides	Emulsifier; dispersant; stabilizer.	Disperses and stabilizes pesticides in oil base; disperses pesticides and surfactants in water.
2. Inks	Emulsifier; dispersant; stabilizer; grinding aid.	Improves pigment solubility and flow properties; stabilizes dispersion.
3. Magnetic tape	Emulsifier; dispersing agent; antioxidant.	Improves dispersion and shelf-life.
4. Lacquers, paints and other coatings	Hydrophilic emulsifying and wetting agent; dispersing agent; stabilizer; antioxidant.	Improves dispersion of pigments, stability and shelf-life.
5. Leather	Softening agent; oil penetrant.	Improves the process of fat-liquoring.
6. Plastics	Emulsifier; dispersing agent; releasing agent.	Improves dispersion of pigments and mold release.
7. Rubber	Emulsifier; dispersing agent; releasing agent; antioxidant.	Improves dispersion of pigments, mold release and shelf-life.
8. Textiles	Softening agent; lubricant; pigment disperser.	Specific properties desired; depend on concentrations applied.
9. Drilling oil	Emulsifier; dispersing agent.	Facilitates viscosity adjustment.

TABLE VII

Phospholipid Content of Select Seeds and Seed Products (%)

Phospholipid	Cottonseed					Soybean		Oats (18)	Ginkgo nuts (19)	Barley[a] (20)	Castor bean (16)	Peanut (16)	Pine kernel (16)	Winter wheat[a] (21)
	I (16)[b]	II (8)	Hydraulic press (8)	Solvent extract (8)	Screw press (8)	I (16)	Azolectin (commercial) (17)							
Phosphatidylcholine (PC)	33	28.6	—	—	—	45	35.2	29.9	21.5	44.4	13	49	54	45.4
Phosphatidylethanolamine (PE)	22	19.3	37.2	38.1	38.4	15	28.5	14.8	23.9	8.2	83	16	11	—
Phosphatidylserine (PS)	—	22.0	32.6	31.1	31.0	—	—	3.2	—	4.9	—	—	—	3.3
Phosphatidylinositol (PI)	37	10.1	6.4	6.4	6.2	25	18.3	—	—	1.2	trace	22	30	—
Phosphatidylglycerol (PG)	—	—	—	—	—	—	—	—	52.1	0.5	—	—	—	—
Diphosphatidylglycerol (DPG)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PG + DPG	—	—	—	—	—	—	8.5	—	—	1.6	—	—	—	—
Phosphatidic acid (PA)	—	—	—	—	—	—	—	—	—	0.1	—	—	—	trace
PA + PI	—	—	—	—	—	—	—	3.9	—	—	—	—	—	—
Lysophosphatidylcholine (LPC)	—	6.2	10.1	10.0	10.2	—	—	—	1.0	37.0	—	—	—	14.0
Lysophosphatidylethanolamine (LPE)	—	—	—	—	—	—	—	—	1.4	—	—	—	—	1.6
Lysophosphatidylserine (LPS)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PC + LPE + LPS	—	—	—	—	—	—	—	20.4	—	—	—	—	—	—
LPE + Lysophosphatidic acid	—	—	—	—	—	—	—	19.4	—	—	—	—	—	11.6
N-acylysophosphatidylethanolamine	—	—	—	—	—	—	—	—	—	—	—	—	—	13.2
N-acylphosphatidylethanolamine	—	—	—	—	—	—	—	—	—	—	—	—	—	11.0
Unknown	8	13.8	13.7	14.3	14.1	15	9.6	—	—	2.1	4	13	5	—

[a]Expressed as % of total phosphorus.

[b]In Tables I through IX, numbers in parentheses coincide with those in References.

provides an opportunity to produce food-grade cottonseed phospholipids as a by-product of the production of edible oil. This would make the processing of glandless cottonseed oil more economically attractive by increasing revenue, decreasing waste disposal costs, and reducing emulsion problems during refining.

The fatty acid composition of cottonseed oils extracted from glanded and glandless cottonseed from various cultivars are summarized in Table IX. The values of both sources are similar, which would be reflected in the fatty acid composition of the phospholipids.

Cottonseed phospholipids are superior to those of soybean since none of the fatty acids present contain more than 2 double bonds, making them more stable to oxidation and rancidity. Soybean phospholipids contain high amounts of the 3 double-bonded unsaturated linolenic acids that cause flavor, color, and odor problems that should not occur with phospholipids from glandless cottonseed.

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TABLE VIII

Composition of the Acetone Precipitate of Crude Cottonseed Oil (8)

Component	Crude phospholipids[a]	Phosphatidylcholine[b]	Phosphatidylethanolamine[b]	Phosphatidylserine[b]
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]				
8:0	–	–	0.4	0.3
12:0	–	–	0.4	0.4
14:0	0.4	0.3	0.4	0.6
16:0	32.9	31.1	33.7	33.3
16:1	0.5	0.3	0.3	0.6
18:0	2.7	2.8	2.2	0.3
18:1	13.6	11.0	11.5	14.4
18:2	50.0	54.0	49.0	50.4
Unknown	–	–	2.3	–
Saturated fatty acids	36.0	34.2	37.1	34.9

[a]Percentage of crude oil.

[b]Percentage of crude phospholipids.

[c]Fatty acid is indicated by carbon number and the number of double bonds.

TABLE IX

Composition of Fatty Acids in Cottonseed Oils from 16 Cultivars[a] (26)

Fatty acid (%)	Glandless seed varieties								
	A	B	C	D	E	F	G	H	Mean
Myristic	0.8	0.7	0.7	0.9	0.8	0.7	0.6	0.6	0.7
Palmitic	24.0	20.3	17.6	26.0	24.1	22.1	26.0	20.8	22.6
Stearic	2.1	2.0	2.1	2.4	2.0	1.9	2.4	2.1	2.1
Oleic	16.0	17.1	19.2	17.9	17.3	18.6	16.3	19.2	17.7
Linoleic	56.5	59.4	60.4	52.1	55.8	56.1	54.7	57.0	56.5
Unknown	0.7	0.5	0.0	0.7	0.0	0.6	0.0	0.3	0.4
	Glanded seed varieties								
	A	B	C	D	E	F	G	H	Mean
Myristic	0.8	1.5	0.7	1.0	1.0	0.6	0.8	0.6	0.9
Palmitic	23.9	24.6	24.0	17.6	22.1	22.1	24.5	24.8	23.0
Stearic	2.1	2.2	2.1	2.2	2.0	2.2	2.4	2.5	2.2
Oleic	18.3	18.1	16.3	18.2	16.4	20.7	18.7	15.0	17.7
Linoleic	54.9	52.7	56.4	60.5	58.0	53.9	53.0	57.1	55.8
Unknown	0.0	0.8	0.5	0.5	0.5	0.5	0.6	0.0	0.4

[a]Cottonseeds for this study were obtained from: (a) ACCO Seed, Plainview, TX, (b) Dr. Luther Bird, Texas A&M University, (c) Coker's Pedigreed Seed Company, Lubbock, TX, (d) Dunn Seed Farms, Inc., Lamesa, TX, (e) Gregg Seed Farms, Plainview, TX, (f) Lambright Seed Farms, Slaton, TX, (g) Lockett Seed Company, Vernon, TX, (h) Dr. N.R. Malm, New Mexico State University, (i) U.S. Cotton Research Station, Shafter, CA.

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