Composition of Soybean Lecithin

C. R. SCHOLFIELD, Northern Regional Research Center, Science and Education Administration, Agricultural Research, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

Commercial soybean lecithin is a complex mixture containing ca. 65-75% phospholipids together with triglycerides and smaller amounts of other substances. The major phospholipids include phosphatidylcholine, phosphatidylethanolamine and inositol-containing phosphatides. Other substances reported include carbohydrates, pigments, sterols and sterol glycosides. This paper reviews the nature of the compounds found in soybean lecithin and our present knowledge of its composition.

INTRODUCTION

The term "lecithin" as used today refers to the material obtained by degumming crude vegetable oils and drying the hydrated gums. In the U.S., commercial lecithin is predominantly from soybean oil. The specific phospholipid formerly called lecithin is now referred to as phosphatidylcholine.

Lecithin, then, consists not only of a mixture of phospholipids but also of triglycerides and other nonphospholipid compounds removed from the oil in the degumming process. Composition of soybean lecithin has been considered in many reviews, and substances commonly reported included triglycerides, fatty acids, pigments, sterols, sterol glycosides and esters, tocopherols, and carbohydrates (1-6). Ranges in composition from some of these reviews are in Table I, and values for some other minor components (7) are in Table II. Composition of commercial lecithin is further complicated by the production of six grades of plastic and fluidized lecithin in unbleached, single-bleached and double-bleached forms (2,4,6). In addition, various other refined and modified materials are produced (5,6,8). Little has been published about the chemical nature of changes produced by peroxide bleaching or other modifications. This paper reviews the composition of the phospholipids and other materials extracted with the oil and removed by degumming as determined by studies of commercial and laboratory prepared materials. Wittcoff has discussed soybean phosphatides in his comprehensive book "The Phosphatides" (9).

NONPHOSPHOLIPID COMPONENTS

Carr (10) has stated that oil content may be varied by changing the conditions of centrifugation, and ca. 7% soybean oil and 3% fatty acids are blended with the lecithin to increase fluidity.

Color of soybean lecithin depends on processing and bleaching conditions. At our laboratory (11), we showed that xanthophylls are preferentially removed with the gums and that carotene remains with the oil. Lutein made up about 75% of the carotenoid pigments in the gums. These carotenoid pigments are largely destroyed by peroxide bleaching, leaving a variable amount of brown color with no characteristic absorption bands. Studies by Zuev et al. (12-14) have confirmed the formation of brown-colored substances as well as destruction of carotenoids by heating. They suggest oxidation is involved. Although Burkhardt (15) found in safflower oil that phosphatidylethanolamine contributed most to color formation, Tomioka and Kaneda (16,17) found the brown products similar to those formed in an aldol condensation reaction rather than in a Maillard reaction.

Sterols and sterol glycosides have long been known in soybean lecithin. In addition, Lepage (18) has isolated an esterified form, or acylated steryl glucoside. In a sample of crude soybean lecithin, Kiribuchi et al. (19) reported free sterols 0.5%, steryl glucoside 2.1% and acylated steryl glucoside 2.6%. Steryl ester was present in negligible amounts. In all three forms, campesterol, stigmasterol and β-sitosterol were present in a ratio of ca. 20:30:50. All fatty acids normally in soybean oil were found in the acylated steryl glucosides with increased amounts of saturated acids. Popov et al. (20) found 0.74% free sterol and 2.68% combined steryl glycoside and esterified steryl glycoside.

Although tocopherols are present in soybean lecithin, no recent values were found for their amount. Review articles (2,4,7) list a concentration of ca. 0.1%. One might expect that the proportions of individual tocopherols would be similar to soybean oil; a recent study of soybean oil (21) shows a ratio of α:γ:σ tocopherol of ca. 5:68:27.

These oil-soluble nonphosphatide materials are largely separated from the phospholipids by extracting with acetone. Some sterol glycosides and free carbohydrates, as well as sugars bound to lipid constituents, remain with the acetone-insoluble phospholipids. At our laboratory (22), one sample of oil-free phosphatides was found to contain 7% free sugars made up of 45% sucrose, 9% raffinose and 47% stachyose.

PHOSPHOLIPID COMPONENTS

Each of the phospholipid classes is a mixture of any individual compounds with different fatty acids. Reported fatty acid compositions of total acetone-insoluble phosphatides vary considerably. Some values are listed in Table III. Although the fatty acids of phosphatide classes differ, little has been published on pure materials isolated so as to preclude fractionation. Another complexity is added to

TABLE I

Reported Range of Components of Soybean Lecithin (1–6)

	%
Phosphatidylcholine	19–21
Phosphatidylethanolamine	8–20
Inositol phosphatides	20–21
Other phosphatides	5–11
Soybean oil	33–35
Sterols	2–5
Carbohydrates, free	5
Moisture	1

TABLE II

Some Minor Components of Soybean Lecithin (7)

Tocopherol	1.3	mg/g
Biotin	0.42	μg/g
Folic acid	0.60	μg/g
Thiamin	0.115	μg/g
Riboflavin	0.33	μg/g
Panothenic acid	5.59	μg/g
Pyridoxine	0.29	μg/g
Niacin	0.12	μg/g

TABLE III

Some Reported Fatty Acid Compositions of Soybean Phosphatides

	Hilditch and Zaky (23) (%)	Vijayalakshime and Rao (24) (%)	Daga (25) (%)
14:0	–	–	1.9
14:1	–	–	tr
16:0	11.7	42.7	26.7
16:1	8.6	7.0	–
18:0	4.0	11.7	9.3
18:1	9.8	17.0	25.1
18:2	55.0	20.0	27.0
18:3	4.0	1.6	–
20:0	1.4	–	–
Unsaturated C_{20-22}	5.5	–	–

phosphatide composition by the different cations associated with acidic groups. Potassium, sodium, magnesium and calcium have been reported (2).

Of the phospholipids in soybean lecithin, the best known is phosphatidylcholine (PC), for which the structure is shown in Figure 1. This formula represents the α isomer. Hydrolysis of PC results in both α and β glycerophosphoric acid, and this was formerly considered evidence for both α and β PC. However, Baer (26) has shown that hydrolysis of the synthetic compounds L-α-glycerylphosphorylcholine, L-α-glycerylphosphorylethanolamine, L-α-PC, or L-α-phosphatidylethanolamine (PE) all yield mixtures of α- and β-glycerophosphoric acid that resemble closely those reported from natural PC. He states that the concept of the occurrence of β-PC or β-PE in nature is no longer tenable. Privett and Blank (27) and Mangold (28), by ozonolysis of PC and thin layer chromatography of the resulting aldehyde "core," have shown four fatty acid groups in PC: α-saturated-β-unsaturated, α-unsaturated-β-saturated, α-β-unsaturated, and a small amount of α-β-saturated. Kimura et al. (29) by $AgNO_3$-TLC separated soybean PC into four fractions, including one containing 64% arachidonic acid. Recently, Crawford et al. (30) by high pressure reverse-phase chromatography have isolated fractions from soybean PC ranging in unsaturation from dilinolenyl to stearyl, linolyl PC.

In older literature, the term "cephalin" has been used for PE (Fig. 1) and as "cephalin fraction" for material insoluble in alcohol. Actually, soybean PE has been found both in the alcohol and alcohol insoluble fractions (31-33), and a 30 transfer countercurrent distribution of the alcohol-soluble fraction between hexane and 90% methanol resulted in only partial separation of PE from the completely alcohol-soluble PC. Kimura et al. (34), by methods like they used with PC, also found arachidonic acid in soybean PE.

Closely related to PE is phosphatidylserine (PS), found in soybean lecithin by Van Handel (35). Negishi et al. (36) reported 5.9% PS in a commercial soybean lecithin and Nielson (37) found PS in difficultly extractable soybean phosphatides.

The structure of N-acylphosphatidylethanolamine (NPE) in soybean lecithin was determined by Aneja et al. (38). Wilson and Rinne (39) found it to be more abundant in developing seed and to decrease at maturity.

Phosphatidic acid (PA), the moiety common to all the glycerophosphatides, is present in soybean lecithin (1,2,5). It, also, has been found in greater amounts of lipids from developing than from mature seeds (39, 40). Both PA and NPE are decreased by extraction procedures that inactivate enzymes (41, 42). The amount of NPE and PA in lecithin, therefore, may be dependent on enzymatic reactions during storage and processing of the beans, and PA as calcium and magnesium salts also remains in the degummed oil as nonhydratable phosphatides (43, 44).

Inositol was first found in soybean phosphatides by Klenk and Sakai (45), and early work was done by Woolley (46) and Folch (47). At our laboratory, the inositol lipids in soybean phosphatides were separated into two fractions by countercurrent distribution between hexane and 95% methanol (31). The fraction of lower partition coefficient—more soluble in methanol—was found to contain PE and an inositol-containing phosphatidic acid (32). This inositol compound corresponds to phosphatidylinositol (PI) for which the structure was determined by Okuhara and Nakayama (48). Its absolute configuration was later shown to be as in Figure 1 (49). The fraction of higher partition coefficient—more soluble in hexane—contained, in addition to inositol, the sugars galactose, mannose and arabinose (22).

These complex glycolipids and similar materials from other sources have been very difficult to purify and characterize. They were the object of much work by Carter and his group, and a list of his papers on this and related subjects has been published (50). Van Handel (35, 51) found evidence for a long chain base in soybean phosphatides, and Carter et al. (52,53) identified phytosphingosine and dehydrophytosphingosine (1,3,4-trihydroxy-2-amino-octadecane and 1,3,4,-trihydroxy-2-amino-8-*trans*-octadecene) in soybean phosphatides in the ratio saturated:dehydro of 20:80.

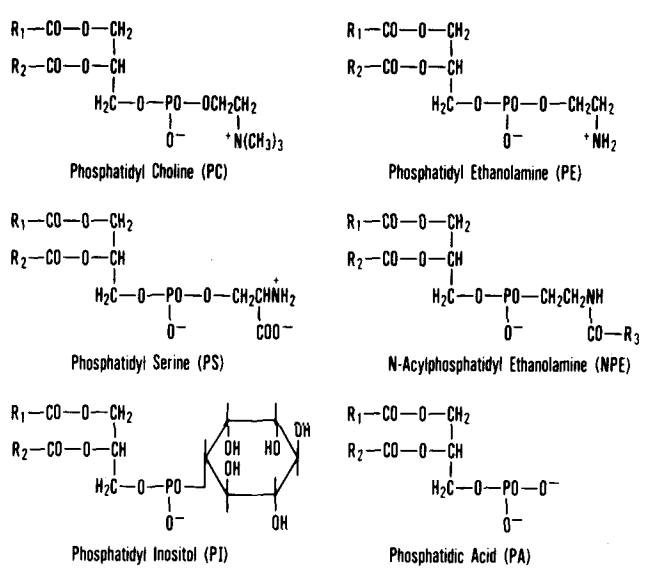

FIG. 1. Some phosphatides in soybean lecithin.

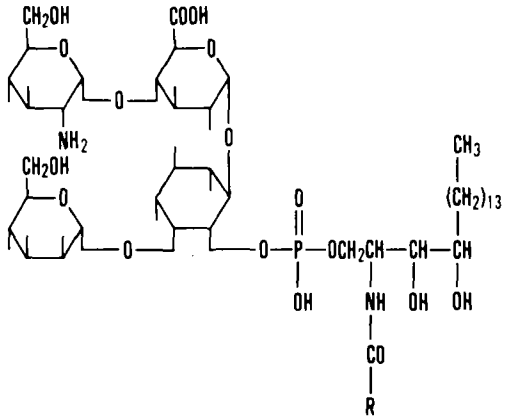

FIG. 2. Phytoglycolipid tetrasaccharide from phospholipids.

In a series of papers (54-59), the complete structure of a phytoglycolipid (PGL) tetrasaccharide containing phytosphingosine, fatty acid, phosphorus, inositol, mannose, hexuronic acid and glucosamine was determined to be as shown in Figure 2. To this structure, arabinose, galactose and a small amount of fucose is attached through glucosamine to give various chain lengths, including octa- and higher oligosaccharides (57,58). Soybean PGL may be presumed to be mainly the unsaturated analog of the structure just discussed (53). In soybean PGL, the fatty acid composition was cerebronic acid 5% and palmitic plus stearic acids 95%.

Isolation of PGL involved alkaline hydrolysis of phospholipids and did not preclude possible cleavage of a more polar moiety (54). Purification by countercurrent distribution was unsatisfactory because impurities were bound as mixed chelated calcium and magnesium salts. This difficulty was overcome by passage of the lipids through a chelating resin column (60). Using countercurrent distribution of soybean phospholipids in the sodium form, Carter and Kisic (61) confirmed the presence of PGL as previously isolated by alkaline hydrolysis, and also found a second glycolipid, called ceramide-phosphate polysaccharide, devoid of hexosamine but containing inositol, hexuronic acid, galactose, arabinose, fucose and mannose. They also found a material, lipophytin, high in phosphorus and insoluble in their hexane, butanol, methanol, water solvent system. These last two substances still do not appear to have been further characterized.

Modern chromatographic methods have made possible the detection of many other materials in soybean lecithin. Although the major phospholipids are PC, PE and PI, many other components are present in small amounts. Szuhaj et al. (62), by silicic acid column and thin layer chromatography, found 17 classes as follows: triglycerides, diglycerides, monoglycerides, free fatty acids, free sterols, steryl esters, diphosphatidylglycerol, PE, PC, PI, lyso-PC, esterified steryl glucoside, steryl glucoside, digalactosyldiglycerides, cerebrosides and two unidentified glycolipids. Erdahl et al. (63) found most of the above and also NPE, lyso-PE, PS, phosphatidylglycerol and eight unidentified components.

REFERENCES

1. Daubert, B. F. in "Soybeans and Soybean Products," edited by K.S. Markley, Vol. I, Interscience Publishers, Inc., New York, 1950, pp. 185–192.
2. Stanley, J., Ibid., Vol. II, 1951, pp. 593–647.
3. Iveson, H. T., Soybean Dig. 21:18 (June 1961).
4. Sartoretto, P., in "Kirk Othmer Encyclopedia of Chemical Technology," 2nd Edition, Vol. 12, Interscience Publishers, New York, 1967, pp. 343–361.
5. Van Nieuwenhuyzen, W., JAOCS 53:425 (1976).
6. Wolf, W.J., and D.J. Sessa in "Encyclopedia of Food Science," edited by M.S. Peterson and A.H. Johnson, AVI Publishing CO., Inc., Westport, CT, 1978, pp. 461–467.
7. Chang, S. S., and J.R. Wilson, Ill. Med. J. 675 (1964).
8. Sullivan, D.R., and B.F. Szuhaj, JAOCS 52:Program Abstract 39, 66th annual meeting, AOCS (April 1975).
9. Wittcoff, H., "The Phosphatides," Reinhold Publishers Corp., New York, 1951, p. 219.
10. Carr, R.A., JAOCS 53:347 (1976).
11. Scholfield, C.R., and J.H. Dutton, Ibid. 31:258 (1954).
12. Zuev, E.I., V.V. Klyuchkin and V.P. Rzhekhin, Tr VNII Zhirov 27:117 (1970); CA. 76:5780lk (1972).
13. Klyuchkin, V.V., E.I. Zuev and V.L. Loseva, Ibid. 27:127 (1970); C.A. 76:57818w (1972).
14. Zuev, E.I., and V.V. Klyuchkin, Ibid. 27:136 (1970); C.A. 76:57819x (1972).
15. Burkhardt, H.J., JAOCS 47:69 (1970).
16. Tomioka, F., and T. Kaneda, Yukagaku 23:777 (1974).
17. Tomioka, F., and T. Kaneda, Ibid. 23:782 (1974).
18. Kepage, M., J. Lipid Res. 5:587 (1964).
19. Kiribuchi, T., M. Takemitsu and F. Saburo, Agric. Biol. Chem. 30:770 (1966).
20. Popov, A., Ts. Milkova and N. Marekov, Nahrung 19:547 (1973).
21. Carpenter, A.P., Jr., JAOCS 56:668 (1979).
22. Scholfield, C.R., H.J. Dutton and R.J. Dimler, Ibid. 29:293 (1952).
23. Hilditch, T.P., and Y.A.H. Zaky, Biochem. J. 36:815 (1942).
24. Vijayalakshmi, B., and S.V. Rao, Chem. Phys. Lipids 9:82 (1972).
25. Daga, H.G.M., Indian Chem. J. 11:17 (1976); C.A. 85: 190977Z (1976).
26. Baer, E., JAOCS 42:257 (1965).
27. Privett, O.S., and M.L. Blank, Ibid. 40:70 (1963).
28. Mangold, H.K., Ibid. 38:708 (1961).
29. Kimura, S., M. Motoki and K. Shibasaki, Nippon Shokuhin Kogyo Gakkai Shi 16:425 (1969); C.A. 73:129716z (1970).
30. Crawford, C.G., R.D. Plattner, D.J. Sessa and J.J. Rackis, Lipids 15:91 (1980).
31. Scholfield, C.R., H.J. Dutton, F.W. Tanner, Jr., and J.C. Cowan, JAOCS 25:368 (1948).
32. Scholfield, C.R., and H.J. Dutton, J. Biol. Chem. 208:461 (1954).
33. Scholfield, C.R., and H.J. Dutton, Ibid. 214:633 (1955).
34. Kimura, S., and K. Shibasaki, Nippon Shokuhin Kogyo Gakkai Shi 16:369 (1969); C.A. 73:129715y (1970).
35. Van Handel, E., "The Chemistry of Phosphoaminolipids," D.B. Centen's Uitgevers-Maatschappij N.V. Amsterdam, 1954.
36. Negishi, T., H. Hayashi, S. Ito and Y. Fujino, Obihiro Chikusandaigaku Gakujutsu Kenkyu Hokuku 5:97 (1967); C.A. 68:56722h (1968).
37. Nielsen, K., JAOCS 37:217 (1960).
38. Aneja, R., J.S. Chadha and J.A. Knaggs, Biochem. Biophys. Res. Commun. 36:401 (1969).
39. Wilson, R.F., and R.W. Rinne, Plant Physiol. 54:744 (1974).
40. Privett, O.S., K.A. Dougherty, W.L. Erdahl and A. Stolyhwo, JAOCS 50:516 (1973).
41. Roughan, P.G., C.R. Slack and R. Holland, Lipids 13:497 (1978).
42. Phillips, F.C., and O.S. Privett, Ibid. 14:949 (1979).
43. Hvolby, A., JAOCS 48:503 (1971).
44. Mounts, T.L., G.R. List and A.J. Heakin, Ibid. 56:883 (1979).
45. Klenk, E., and R. Sakai, Z. Physiol. Chem. 258:33 (1939).
46. Woolley, D.W., J. Biol. Chem. 147:581 (1943).
47. Folch, J., Fed. Proc. 6:252 (1947).
48. Okuhara, E., and T. Nakayama, J. Biol. Chem. 215:295 (1955).
49. Ballou, C.E., and L.I. Pizer, J. Am. Chem. Soc. 82:3333 (1960).
50. Carter, H.E., Collection of papers on the Chemistry and Metabolism of Sphingolipids, edited by C.C. Sweeley, Chem. Phys. Lipids 5:298 (1970).
51. Van Handel, E., Rec. Trav. Chim. 72:763 (1953).
52. Carter, H.E., W.D. Celmer, W.E.M. Lands, K.L. Mueller and H.H. Tomizawa, J. Biol. Chem. 206:613 (1954).
53. Carter, H.E., and H.S. Hendrickson, Biochemistry 2:389 (1963).
54. Carter, H.E., W.D. Celmer, D.S. Galanos, R.H. Gigg, W.E.M. Lands, J.H. Law, K.L. Mueller, T. Nakayama, H.H. Tomizawa and E. Weber, JAOCS 35:335 (1958).
55. Carter, H.E., R.H. Gigg, J.W. Law, T. Nakayama and E. Weber, J. Biol. Chem. 233:1309 (1958).
56. Carter, H.E., D.S. Galanos, H.S. Hendrickson, B. Jann, T. Nakayama, Y. Nakazawa and B. Nichols, JAOCS 39:107 (1962).
57. Carter, H.E., S. Brooks, R.H. Gigg, D.R. Stroback and T. Suami, J. Biol. Chem. 239:743 (1964).
58. Carter, H.E., B.E. Betts and D.R. Stroback, Biochemistry

3:1103 (1964).
59. Carter, H.E., D.R. Stroback and J.N. Hawthorne, Ibid. 8:383 (1969).
60. Carter, H.E., and E.J. Weber, Lipids 1:16 (1966).
61. Carter, H.E., and A. Kisic, J. Lipid Res. 10:356 (1969).
62. Szuhaj, B.F., I.L. Balazs and L.D. Williams, JAOCS 47:Program Abstract 175, 44th fall meeting, AOCS (July 1970).
63. Erdahl, W.L., A. Stolyhwo and O.S. Privett, Ibid. 50:513 (1973).

[Received November 17, 1980]

Effect of Degumming Conditions on Removal and Quality of Soybean Lecithin

G.R. LIST, J.M. AVELLANEDA[1] and **T.L. MOUNTS**, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

A commercially extracted crude soybean oil (570 ppm phosphorus, 1.74% acetone insolubles) was degummed in the laboratory under a wide range of reaction conditions (water concentration, temperature, time and agitation). The reaction conditions were correlated with phosphorus removal from the oil as well as with color and acetone-insoluble content of the gum fraction. Efficiency of removal of phosphorus-containing compounds was independent of time, temperature and agitation. Water concentration had the most significant effect on removal of phosphorus from crude soybean oil. Some darkening of the lecithin was observed at temperatures above 60 C and with increased agitation. Individual conditions of time and temperature had relatively little effect on the acetone-insoluble content of the gums. Low agitation rates and water in concentrations of other than 2% (either more or less) entrained excessive amounts of oil in the gums. Under our experimental conditions, the optimal conditions with respect to phosphorus removal, lecithin color and acetone-insoluble content are estimated to be: time—short (15 min); agitation—moderate to rapid (400 rpm); temperature—60 C; water concentration—2% or an amount close to the phosphatide content of the crude oil. Bleaching with hydrogen peroxide to produce single-bleached lecithin was investigated. From limited data, it appears that when degumming and bleaching are performed simultaneously, effectiveness of bleaching is a function of peroxide concentration and time. Thus, longer degumming times are required to prepare bleached lecithin compared to unbleached products.

INTRODUCTION

Soybean oil is the only commercial source of lecithin, and worldwide production amounts to 100,000 tons/year (1,2). Industrially, lecithin is recovered by treating the crude oil with water; under these conditions the gums are precipitated from solution, separated by centrifugation, and finally dried (2-5). Conditions affecting the yield and quality of lecithin are largely undefined in the literature, particularly when degumming is carried out by agitating the oil in a tank. Factors affecting the efficiency of continuous centrifugal degumming have been reported (6). Oils degummed under commercial conditions typically contain 5-20% of their original phosphatides as measured by their elemental phosphorus contents (7). Thus, a more complete understanding of the degumming process could lead to higher yields and improved lecithin quality.

EXPERIMENTAL—PROCEDURES

Crude Oil, Analytical Methods

A commercially extracted crude soybean oil was used throughout the investigation. It contained 570 ppm phosphorus and 1.74% acetone insoluble (AI) as determined by official AOCS methods CA 12-55 and Ja-4-46, respectively. The equivalent phosphatide (acetone insoluble) calculates to 1.81% (0.0570 × 31.7) (8) and agrees well with the observed value.

Degumming

Crude soybean oil (2,000 g) was charged into a 3-L round-bottomed flask fitted with a stirring shaft and a paddle-shaped Teflon impeller 7.5 cm long driven by a variable-speed motor. The oil was purged with nitrogen through a sinter glass stick for 2 min and brought to the desired temperature under a nitrogen blanket; then the motor was started, and the desired amount of distilled water was added. When degumming was completed, the mixture was cooled to 40 C and the gums were separated by centrifugation at 1,900 rpm for 15 min. The degummed oil was removed by decantation.

Removal of Water from Crude Gums

To characterize the composition of the crude gums and to isolate lecithin without forming additional color bodies, a method was needed to remove the hydration water from the crude gums. Several methods were investigated. The first method consisted of partitioning the crude gums between hexane and ethanol according to the following procedure: crude gums (ca. 20 g) were weighed into a 250-mL Erlenmeyer flask along with an equal weight of hexane. With magnetic stirring, absolute ethanol was then added from a buret until a distinct phase separation occurred. After transfer of the contents to a separatory funnel, the lower layer, consisting of water and ethanol, was discarded. The upper layer (lecithin, oil, color bodies, hexane) was freed of solvent at 30 C under vacuum. The second method consisted of driving water from the crude gums by vacuum stripping (2 hr, 60 C, 28 in. water) on a rotating evaporator. A comparison of these methods is shown in Table I. Although vacuum stripping did not increase color bodies in the lecithin, water removal was low and variable (57-74%) compared to ca. 96% for the partition method. For this reason, the partition method was used throughout the study.

Single-Bleached Lecithins

Single-bleached lecithin was prepared according to the foregoing degumming procedure except that the desired amount of hydrogen peroxide (30% in water, Fisher ACS) was added to the water used for degumming.

[1] Present address, LaPolar, Inc., Obregon, Mexico.