Preferential Degradation of Noncholine Phosphatides in Soybean Lecithin by Thermalization

John D. Weete*
Department of Botany and Microbiology, Auburn University, Auburn, Alabama 36849

Purified soybean lecithin and the gum derived from soybean oil processing were heated separately in bulk at 125 to 200°C for 60 min, or at 175°C for 30, 60, 90 and 120 min, and the products were analyzed by thin-layer chromatography and high-performance liquid chromatography. It was found that the noncholine phosphatides are preferentially degraded relative to phosphatidylcholine, and that these phosphatides are broken down in the order phosphatidylethanolamine (PE) > phosphatidylinositol (PI) > phosphatidic acid (PA) with increasing temperature. At 175°C, the heating time required to degrade the noncholine phosphatides was between 30 and 60 min. Diglycerides were the principal products of thermalization at 77% of the total material, indicating that the 3-phosphoester linkage is the most heat-labile portion of the noncholine phosphatide molecules. Cleavage of the fatty acids from positions 1 and 2 of the phosphatides was minimal, as indicated by the relatively low amount of free fatty acids (8% of the total) when the lecithin was heated at 180°C for 90 min. The appearance of brown discoloration, characteristic of heated lecithin, coincided mainly with the decomposition of PE.

KEY WORDS: Diglycerides, gum, lecithin, phosphatidylcholine, thermalization.

Commercially, the term "lecithin" refers to a wide variety of products that have phosphatides as the sole or major components. Lecithins have multiple uses, such as in foods and beverages, cosmetics, industrial coatings and in animal health and nutrition products (1). The primary function of lecithin in many applications is as an emulsifier.

Phosphatidylcholine (PC) is a major component of lecithins and may be accompanied by other phosphatides, such as phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA) in soybean lecithin (2). PC is becoming increasingly commercially important because of its use in liposomes, which are used in cosmetics and are being developed as drug delivery systems by the pharmaceutical industry (3). Although lecithin as a mixture of phosphatides will form liposomes, PC, particularly the fully acyl-saturated form, is preferred.

It is well-known that exposure to heat causes browning and overall deterioration of the desirable properties of lecithin (4). Nevertheless, it was discovered in this laboratory that heating lecithin under certain conditions of time and temperature greatly enhances its properties as an emulsifier for water-in-oil emulsions (5–7). Analytical studies conducted to determine the chemical changes responsible for the improvement in the emulsification properties of lecithin by thermalization led to the discovery that the noncholine phosphatides were selectively degraded, leaving PC as the sole phosphatide. This communication reports the results of these analytical studies and, in addition, shows that the crudest and least expensive form of lecithin, gum, can be thermalized in a continuous-flow process. It is also shown that PC can be isolated in pure form from the thermalized material by conventional techniques.

*Address correspondence at Nuclear Science Center, Auburn University, Auburn, AL 36849.

EXPERIMENTAL PROCEDURES

Lecithins. Two forms of soybean lecithin were used in this study: granular lecithin at 95–98% phosphatides (Centrolex P; Central Soya, Fort Wayne, IN) and gum, which was taken directly from soybean oil processing (Bunge Corporation, Decatur, AL). Water content of the gum was 35.4% as determined by the Karl Fisher procedure, the phosphatide content as acetone-insoluble (AI) material was 47%, and the oil content after centrifugation at 2000 rpm for 10 min was 22% according to high-performance liquid chromatography (HPLC) analysis. The gum used in this study was centrifuged as described prior to use. Phospholipid standards were obtained from Avanti Polar Lipid (Alabaster, AL), and the mono- and diglycerides were obtained from Sigma Chemicals (St. Louis, MO).

Preparation of thermalized lecithin. Unless noted otherwise, batches (100 g) of lecithin or gum in 1000-mL beakers were heated in bulk in a forced-air oven at 180°C for 90 min, or for times and temperatures as indicated. In addition, a continuous-flow thermalization process was used whereby soybean gum that had been mixed with hexane (1.2:1, vol/vol) and heated (60°C) was passed through a glass 6″ Pope thin-film evaporator (Menomonee Falls, WI) at 200°C with a flow rate of about 15 mL per min. Products of bulk or continuous thermalization are herein referred to as thermalized lecithin or gum, respectively, or collectively as thermalized lecithins.

Fractionation. Thermalized lecithins were partially fractionated by separating the acetone-soluble (AS) and AI components. Acetone was added to the thermalized lecithin (4:1, wt/vol). The mixture was stirred by vortexing and then allowed to set at room temperature for 4 h. The AI fraction was collected by centrifugation at 2000 rpm, washed with acetone and then air-dried. The solvent was removed from the AS fraction by rotary flash evaporation.

The AI material was further fractionated into methanol or ethanol-soluble (ES) and ethanol-insoluble (EI) fractions by the same procedure as that described for fractionation with acetone. Although the efficacy of the two alcohols was not directly compared, there was no noticeable difference between them in precipitating the phosphatides. The ES fraction in 85% ethanol was passed through a 2-cm diameter aluminum oxide column at a 15 to 0.5 ratio (w/w) of adsorbent to residue from the ES fraction. After removal of the solvent from the column eluate, the residue was stored at −70°C or analyzed immediately by HPLC. The procedure was modified from that described by Betzing (8) and Gunther (9).

HPLC. HPLC separation of the components of thermalized and nonthermalized lecithin was conducted essentially by the method of Moreau *et al.* (10) with a Spectra Physics (San Jose, CA) SP-8700 chromatograph linked to a Spectra Physics SP-4270 Integrator as described by

Weete *et al.* (7). The chromatograph was equipped with a LiChrosorb Si 60 chrompack column (10 cm × 0.3 cm, 5 μm) and a Varex Universal (Burtonsville, MD) HPLC evaporative light-scattering detector (ELSD II).

Thin-layer chromatography (TLC). TLC was conducted as described previously (11) for the neutral and polar lipids, which were visualized on the TLC plates with iodine vapor.

Fatty acid analysis. Free fatty acids in the unfractionated and thermalized lecithins were determined by standard titration procedures as described in the American Oil Chemists' Society Method Ca 5a-40 (12) with the following modifications: Samples (1 g) were dispersed in acetone (5 mL) with sonication (20 s), and then centrifuged at 3000 rpm for 10 min. Free fatty acids were titrated in the acetone supernatant with NaOH (0.05N).

RESULTS AND DISCUSSION

The AI fraction of granular lecithin decreased almost linearly when heated from 100 to 200°C for 60 min (Fig. 1A). At 200°C, the AI content was reduced to about 31% of the total material and did not change appreciably at higher temperatures (between 200 to 250°C). Similarly, the AI fraction decreased to about 32% of the total when heated at 180°C for 60 min, and there was little change in the AI content when the lecithin was heated for longer than 60 min (Fig. 1B). The proportion of AI generally varied between 40–50% of the total for different batches of thermalized lecithin when the lecithin was heated at 175–180°C for 60 to 90 min.

We have reported previously (7) that, according to differential scanning calorimetry analysis, there is considerable reaction activity in granular lecithin heated in the 170–210°C temperature ranges. There was a major endothermic peak in the thermogram from 184.03–190.88°C, and endothermic shoulders at about 172 and 180°C, which indicated additional reaction activity. Reaction activity in this range is also generally consistent with the thermal analysis of lecithin reported by Ross *et al.* (13) and coincides with the temperature for optimum batch thermalization of lecithin for improvement in properties as an emulsifier (7).

Granular lecithin contains 95–98% phosphatides, consisting mainly of PC, PE, PI and PA, with relatively minor amounts of other components (Fig. 2). The decrease in acetone-precipitable material, described in Figure 1, can be explained by degradation of mainly the noncholine phosphatides PE, PI and PA, which were essentially removed from lecithin by heating it at 180°C for 90 min, leaving PC as the sole phosphatide (Fig. 3). There was a corresponding increase in the mono- and diglyceride content after thermalization (Fig. 3, Ref. 7).

Granular lecithin was heated for 60 min at temperatures from 125 to 200°C, or at 175°C for 60–120 min, to determine the optimum temperature and time for removing noncholine phosphatides and the conditions for minimizing the degradation of PC. Temperatures up to 150°C had relatively little effect on the phosphatide composition of the lecithin after 60 min. However, most of the PE, PI and PA were absent from the material heated at 175°C for this

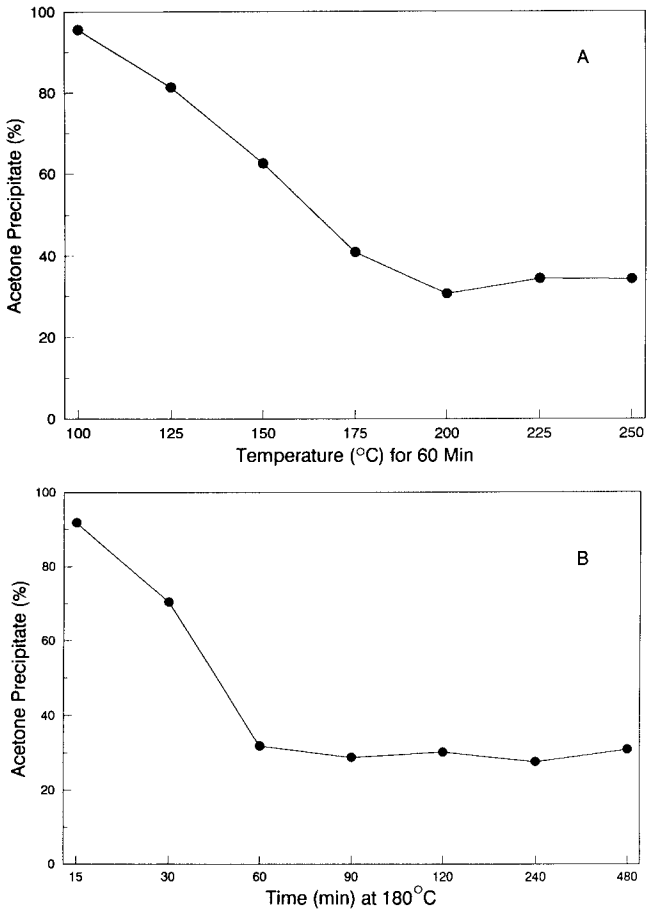

FIG. 1. Change in the acetone-precipitable content of granular lecithin heated in bulk as a function of temperature (A) and time of heating (B). Individual batches (100 g) of lecithin were heated either at 180°C for different time periods, or for 60 min at different temperatures.

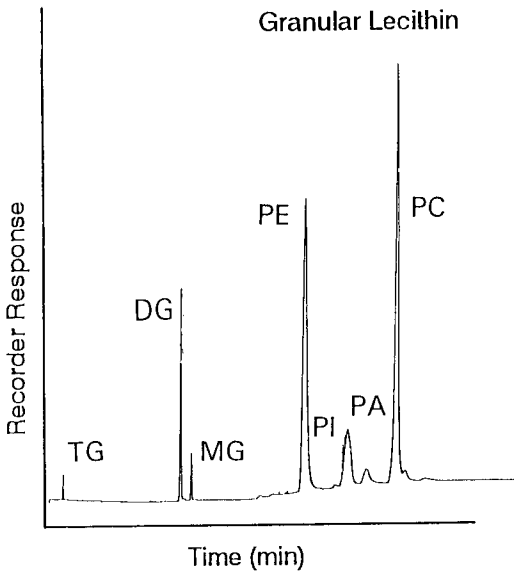

FIG. 2. High-performance liquid chromatographic chromatogram of granular soybean lecithin. TG, triglyceride; DG, diglyceride; MG, monoglyceride; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; and PC, phosphatidylcholine.

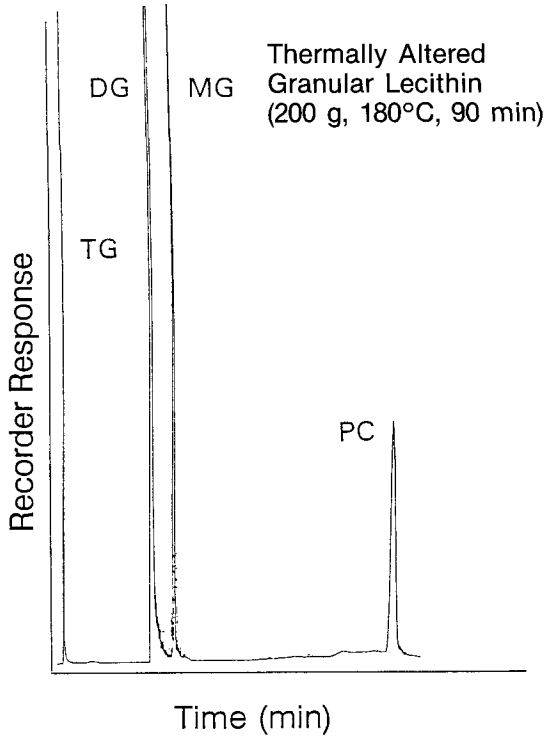

FIG. 3. High-performance liquid chromatographic chromatogram of granular soybean lecithin heated in bulk at 180°C for 90 min. See Figure 2 for abbreviations.

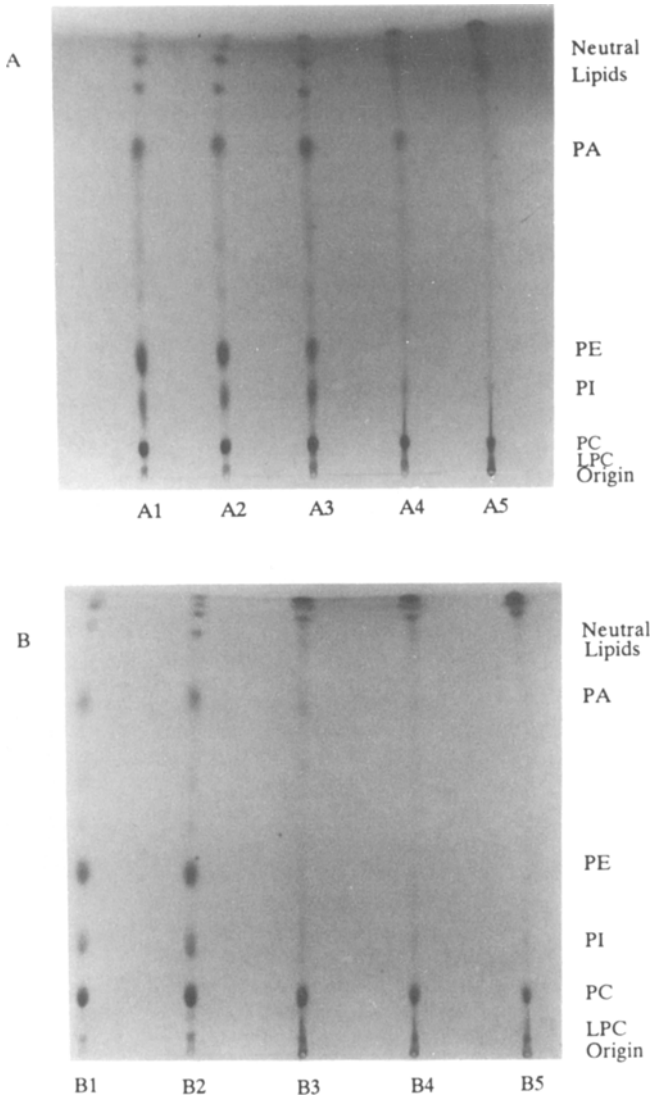

FIG. 4. Thin-layer chromatographic chromatogram of the polar lipids in granular lecithin heated as a function of temperature (A) and time (B) at 175°C. A1, B1, unheated granular lecithin; A2, 125°C; A3, 150°C; A4, 175°C; A5, 200°C; B2, 30 min; B3, 60 min; B4, 90 min and B5, 120 min. Abbreviations as in Figure 2; LPC, lysophosphatidylcholine.

period of time, and virtually none of these phosphatides were detected in the samples heated at 200°C (Fig. 4A).

PI, PE and PA were essentially absent from thermalized lecithin after 60-min heating at 175°C (Fig. 4B). Although PC appeared to progressively decrease in amount with time, a substantial portion remained after heating for 120 min. Degradation of the noncholine phosphatides coincided with the appearance of neutral lipid that can be seen at the TLC solvent front, and a polar substance(s) that remained at the TLC plate origin (Fig. 4). This experiment has been repeated several times with essentially the same results. However, the timing at which compositional changes occur in bulk thermalization is dependent on the amount of material being thermalized, as well as on the temperature.

TLC analysis of the thermalized lecithin showed that mono- and diglycerides (1,2 and 1,3 isomers) and free fatty acids increased progressively with increasing temperature, beginning at 150°C for 60 min (Fig. 5A) and from 30 to 120 min heating at 175°C (Fig. 5B). These substances were in the AS fraction, with the diglycerides being the major components at 77% of the fraction. Minor amounts of triglycerides and monoglycerides were also present in the AS fraction (Fig. 5) and, according to HPLC analysis, were accompanied by PC (data not given). The free fatty acids did not elute from the HPLC column with the solvent system used and are therefore not shown in the chromatograms in Figure 3.

As we have reported previously (7), the main phosphatide degradation products were diglycerides at 77% of the AS fraction. In this study, the AI fraction of thermalized lecithin was subjected to further fractionation by methanol. A considerable portion (40–50%) of the AI fraction was a black, alcohol-insoluble, coal-like solid, and the methanol-soluble fraction contained 96% PC and 4% monoglycerides (Table 1).

The black alcohol-insoluble material is believed to be a polymer formed from the phosphatide degradation products. Tomioka and Kaneda (14–17) suggested that the brown material is a polymer that retains the structure of the original lecithin. However, the accumulation of relatively large amounts of diglycerides (7) suggests that this may not be the case. Other studies suggest that PE is a major reactant that leads to the darkening of lecithin during heating, i.e., Maillard-type reactions involving amines and aldehydes (18–20). This is supported by results showing that the browning reaction between acetaldehyde and phospholipid was proportional to the amount of PE added to the reaction mixture (21). In this study, the

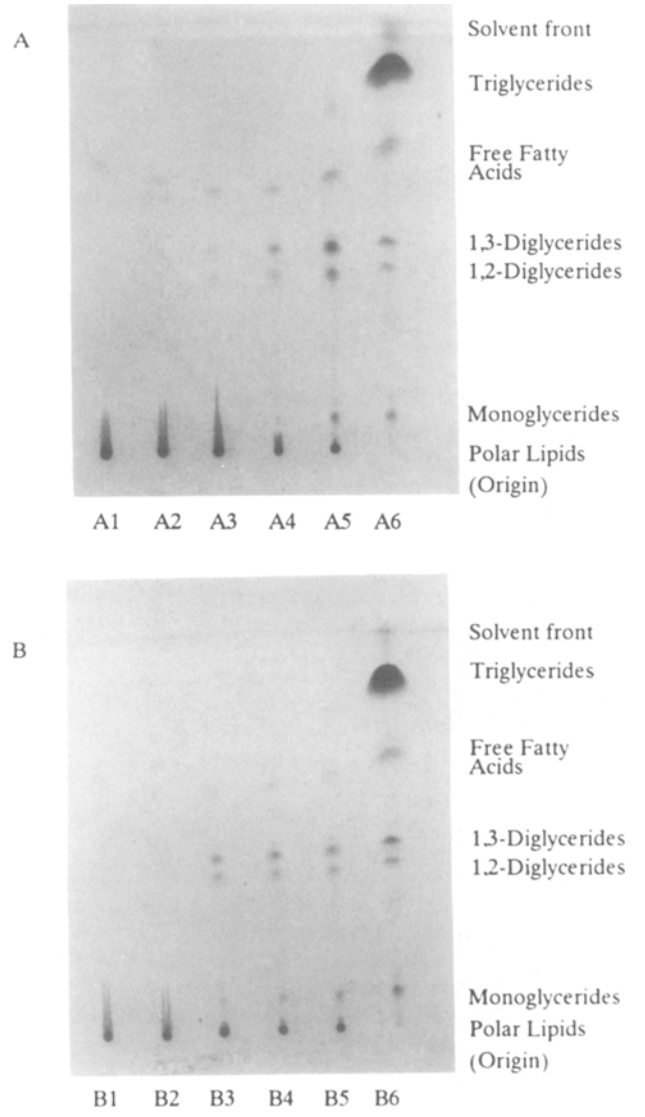

FIG. 5. Thin-layer chromatographic chromatogram of the neutral lipids in granular lecithin heated in bulk as a function of temperature (A) for 60 min and time (B) at 175°C. A1, B1, unheated granular lecithin; A2, 125°C; A3, 150°C; A4, 175°C; A5, 200°C; A6, B6, neutral lipid standard; B2, 30 min; B3, 60 min; B4, 90 min and B5, 120 min.

disappearance of PE from the heated lecithin corresponded to the formation of the brown coloration of the heated lecithin and the presence of a polar brown material, as shown by TLC (origin) (Fig. 4). It is not known how PI and PA react under the thermalization conditions.

Heating lecithin in bulk for periods of 60 to 90 min would not be expected to yield the most uniform product, either as an emulsifier or as a source of PC, because of nonuniform heating of the material. Therefore, a continuous-flow thermalization process was developed with a Pope thin-film evaporator as a reactor. Soybean gum was substituted for granular lecithin to determine if this material could be effectively thermalized and PC recovered in a highly pure form, as described previously. The time of exposure of the lecithin to heat during this process was

TABLE 1

Fractionation of Thermalized Granular Lecithin or Gum from Soybean[a]

Fractions/subfractions	Relative amount (%)	
	Lecithin[a]	Gum[a]
Acetone-soluble	50	52[b]
Phosphatidylcholine	1	—
Triglyceride	3	—
Diglyceride	77	—
Monoglyceride	12	—
Free fatty acids	8	—
Acetone-insoluble	50	48
MeOH- or EtOH-soluble[c,d]	40	57
Phosphatidylcholine	96	99
Triglyceride	4	trace[e]
Diglyceride	—	trace
Monoglyceride	—	trace
Lysophosphatidylcholine	—	trace
MeOH- or ETOH-insoluble[b,c]	60	43

[a]Lecithin was thermalized in bulk at 180°C for 90 min. Gum was thermalized by the continuous-flow process at 200°C.
[b]Not analyzed further.
[c]MeOH was used in the fractionation of lecithin and EtOH for gum.
[d]Analyzed after removal of the dark color by passing it through an aluminum oxide column.
[e]< 1% of the total.

minimized to 20–30 s with the aim of reducing degradation of the PC. Preliminary tests indicated that thermalization could be achieved at 200°C.

Unlike the thermalized granular lecithin, which was a thick, tar-like past at room temperature, the thermalized gum was a liquid. It was fractionated as before to remove the AS and alcohol-insoluble materials, but in this case with ethanol rather than methanol. The results with gum were similar to those with the thermalized granular lecithin, i.e., the AI and AS fractions represented 52 and 48%, respectively, of the total material, and the ES and EI fractions comprised 57 and 43% of the AI fraction, respectively (Table 1). All of the fractions were dark brown. Therefore, the ES fraction was passed through an aluminum oxide column to remove the dark color. The decolorized fraction contained 99% PC with traces of mono-, di- and triglyceride and lysophosphatidylcholine (Fig. 6), and was an amber paste after removal of the solvent.

The fatty acid composition of the gum was not substantially altered during the thermalization process with palmitic, stearic, oleic, linoleic and linolenic acids as the major fatty acids in similar relative proportions in the thermalized and nonthermalized lecithins (7). Linoleic acid was slightly enriched in PC.

In summary, heating lecithin, whether in the highly purified or the most crude forms, under certain conditions of time and temperature leads to the selective removal of noncholine phosphatides, leaving PC as the sole phosphatide in the thermalized material. Patented methods for the large-scale isolation of this phosphatide from crude soybean gum yield materials highly enriched in PC but not completely devoid of PE (8,9). Combined with known fractionation methods, such as acetone and alcohol precipitation and aluminum oxide chromatography, thermalization is a useful pretreatment of lecithin for the subsequent isolation of PC. Thermalization can be achieved in a controlled continuous-flow process, and it

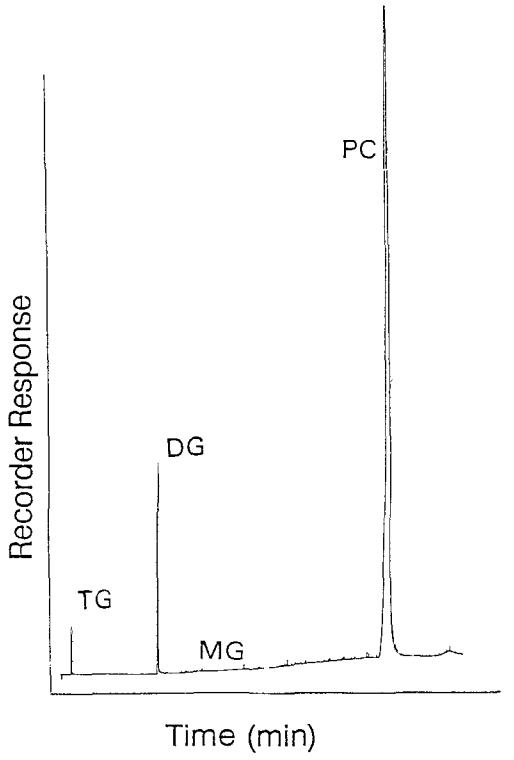

FIG. 6. High-performance liquid chromatographic chromatogram of PC fraction from thermalized gum prepared by continuous-flow thermalization after removal of the brown color by aluminum oxide chromatography. Abbreviations as in Figure 2.

would be expected that this process can be adapted to scale-up production of the thermalized material for use as an emulsifier (7) or as a source of PC.

ACKNOWLEDGMENTS

The author is grateful for the technical assistance of Palmer Stainback and Yao Changbin, and to Ricky Vance of Nelson Brothers, Inc., for performing the Karl Fisher analyses and providing use of the particle size analyzer. This study was supported by a contract from Emulsion Technology, Inc.

REFERENCES

1. Szuhaj, B.F. (ed.), *Lecithins*, American Oil Chemists' Society, Champaign, 1989.
2. Schneider, M., in *Lecithins*, edited by B.F. Szuhaj, American Oil Chemists' Society, Champaign, 1989, p. 109.
3. Kullenberg, F.W. *Ibid.*, p. 237.
4. Scholfield, C.R., *Ibid.*, p. 7.
5. Weete, J.D., and G. Griffith, U.S. Patent #4,943,389 (1990).
6. Weete, J.D., and G. Griffith, U.S. Patent #5,008,037 (1991).
7. Weete, J.D., S. Betageri and G.L. Griffith, *J. Am. Oil Chem. Soc. 71*:731 (1994).
8. Betzing, H., U.S. Patent #4,235,793 (1980).
9. Gunther, B-R., U.S. Patent #4,452,443 (1984).
10. Moreau, R.A., P.T. Asman and H.A. Norman, *Phytochem. 29*:2461 (1990).
11. Weete, J.D., M. Sancholle and C. Montant, *Biochim. Biophys. Acta 752*:19 (1983).
12. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, American Oil Chemists' Society, Champaign, 1993, Method Ca 5a-40.
13. Ross, R.A., R. Lemay and A.M. Takacs, *Can. J. Chem. Eng. 63*:639 (1985).
14. Tomioka, F., and T. Kaneda, *Chem. Abst. 82*:96613 (1975a).
15. Tomioka, F., and T. Kaneda, *Ibid.*:96614 (1975b).
16. Tomioka, F., and T. Kaneda, *Ibid. 86*:42075 (1977a).
17. Tomioka, F., and T. Kaneda, *Ibid. 86*:15426 (1977b).
18. Scholfield, C.R., and H.J. Dutton, *J. Am. Oil Chem. Soc. 31*:258 (1954).
19. Corless, G.A., and L.R. Dugan, *Lipids 5*:846 (1970).
20. Nakanishi, T., and K. Suyama, *Agric. Biol. Chem. 38*:1141 (1974).
21. Suyama, K., and S. Adachi, *Ibid. 43*:1981 (1979).

[Received March 10, 1994; accepted June 11, 1994]