Improvement of Lecithin as an Emulsifier for Water-in-Oil Emulsions by Thermalization

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The various forms (granular, liquid, gum) of lecithin can be heated under certain conditions of time and temperature to greatly improve their properties as emulsifiers for water-in-oil emulsions. Viscosity, discontinuous phase-holding capacity, stability and water retention were greatly enhanced in emulsions containing thermalized lecithins as the emulsifier compared to those prepared with corresponding amounts of nonthermalized lecithins. The improved emulsification properties of the thermalized lecithins appeared to be due, at least in part, to an increase in diglycerides and free fatty acids resulting from the thermal degradation of phosphatides.

KEY WORDS: Diglycerides, emulsifier, gum, lecithin, phosphatides, thermalization, water-in-oil emulsion.

Lecithin from soybean is a mixture of phosphatides that consists mainly of phosphatidylcholine, phosphatidylethanolamine (PE), phosphatidylinositol and phosphatidic acid (1). The purest bulk form of lecithin is a powder or granular material that contains 95–98% phosphatides, but it may be blended with vegetable oil to make a liquid form, fractionated to enrich certain phosphatides, chemically or enzymatically modified, or variously formulated to over 40 different commercially available lecithin products (2). The crudest form of lecithin is a gum composed of hydrated phosphatides derived from the early phases of soybean oil processing (3). Lecithins are used widely in foods and beverages, cosmetics, industrial coatings, and in animal health and nutrition products (4). Although it has multiple uses, lecithin is most commonly used as an emulsifier, mainly for oil-in-water (o/w) emulsions.

Heating leads to discoloration of lecithin and is generally considered to be unfavorable to product quality. However, it has been discovered in this laboratory that heating lecithin under specific conditions greatly improves its properties as an emulsifier for water-in-oil (w/o) emulsions (5,6).

EXPERIMENTAL PROCEDURES

Lecithins. Three forms of soybean lecithin were used in this study—granular lecithin at 95–98% phosphatides (Centrolex P; Central Soya, Fort Wayne, IN), a liquid form (Yelkin TS, Ross and Rowe, Decatur, IL) at 65% phosphatides and 35% soybean oil according to manufacturer specifications, and the gum taken directly from soybean oil processing (Bunge Corporation, Decatur, AL). Water content of the gum was 35.4%, as determined by the Karl Fischer procedure. The phosphatide content as acetone-insoluble (AI) materials was 47%, and the oil content after centrifugation at 2000 rpm for 10 min was 22%, according to high-performance liquid chromatography (HPLC) analysis (see below).

Preparation of thermally altered lecithin. Unless specified otherwise, 200-g batches of the lecithins in 600-mL beakers were heated separately in a forced-air oven at 180°C for 90 min, and are referred to herein as thermalized lecithins. Thermalization could also be carried out as a petroleum-based oil solution, but those data will be published separately. The products of thermalization were stored at room temperature until ready for analysis or used for preparing emulsions without noticeable deterioration of quality.

Absorption of light. The absorption of light by granular lecithin that had been heated at different temperatures and times was measured in chloroform solution at 350 nm with a Beckman DU-65 spectrophotometer (Beckman Instruments, Fullerton, CA). The heated lecithin samples were first diluted to 5 g/10 mL chloroform, but further dilutions were required for the darker samples. Therefore, absorbance is expressed on a relative scale of 0 to 140 (Fig. 1A).

Emulsion preparation. Unless noted otherwise, the discontinuous phase was emulsified into the light mineral oil Klearol (Witco, Houston, TX), which contained a thermalized or nonthermalized lecithin as the emulsifier, by using a common kitchen mixer at the maximum speed setting. Both the continuous (oil) and discontinuous (aqueous) phases were heated to about 60°C prior to mixing. The aqueous phase was slowly added to the oil phase while mixing, which was continued for about 1 min after the two phases were emulsified. After emulsification, the containers were covered with foil, and the emulsions were allowed to cool to room temperature prior to the measurement of properties, which was within 24 h of preparation. Emulsions were prepared in 62.5-g batches and, unless noted otherwise, were composed of 18 mL Klearol (Witco), 0.5 g emulsifier (thermalized or nonthermalized lecithins), 4 g Gulfwax (from local grocery store) and 40 g water.

Emulsion stability. Emulsions (40 mL) were placed in cylindrical plastic containers of 30-mm diameter and allowed to remain undisturbed for 24 h. Gravitational settling of the discontinuous phase was measured as the height of the oil layer on the emulsion surface.

Water loss from emulsions. Emulsion droplets (8 to 10 per slide) on glass microscope slides were weighed and then placed in a PAC 9900 gas exchange system (Data Design Group, San Diego, CA) consisting of a temperature- and humidity-controlled plexiglass chamber. The temperature and relative humidity (RH) were held constant at 28°C and 35%, respectively. After 4 h in the chamber, the slides were re-weighed, and water loss was calculated by difference.

Particle size diameter. Average diameter of discontinuous phase particles (globules) was determined with a Shimadzu Saldi Particle Size Analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD).

Viscosity. Viscosity measurements were made with either a Brookfield RVF-1000 viscometer (Brookfield Engineering Labs. Inc., Stoughton, MA) equipped with a #7 spindle at 20 rpm and were noted at the temperatures or with a Brookfield Digital viscometer Model DV II equipped with cone CP-52. In the latter case, 0.5-mL samples were measured at 2.5 rpm and 25°C.

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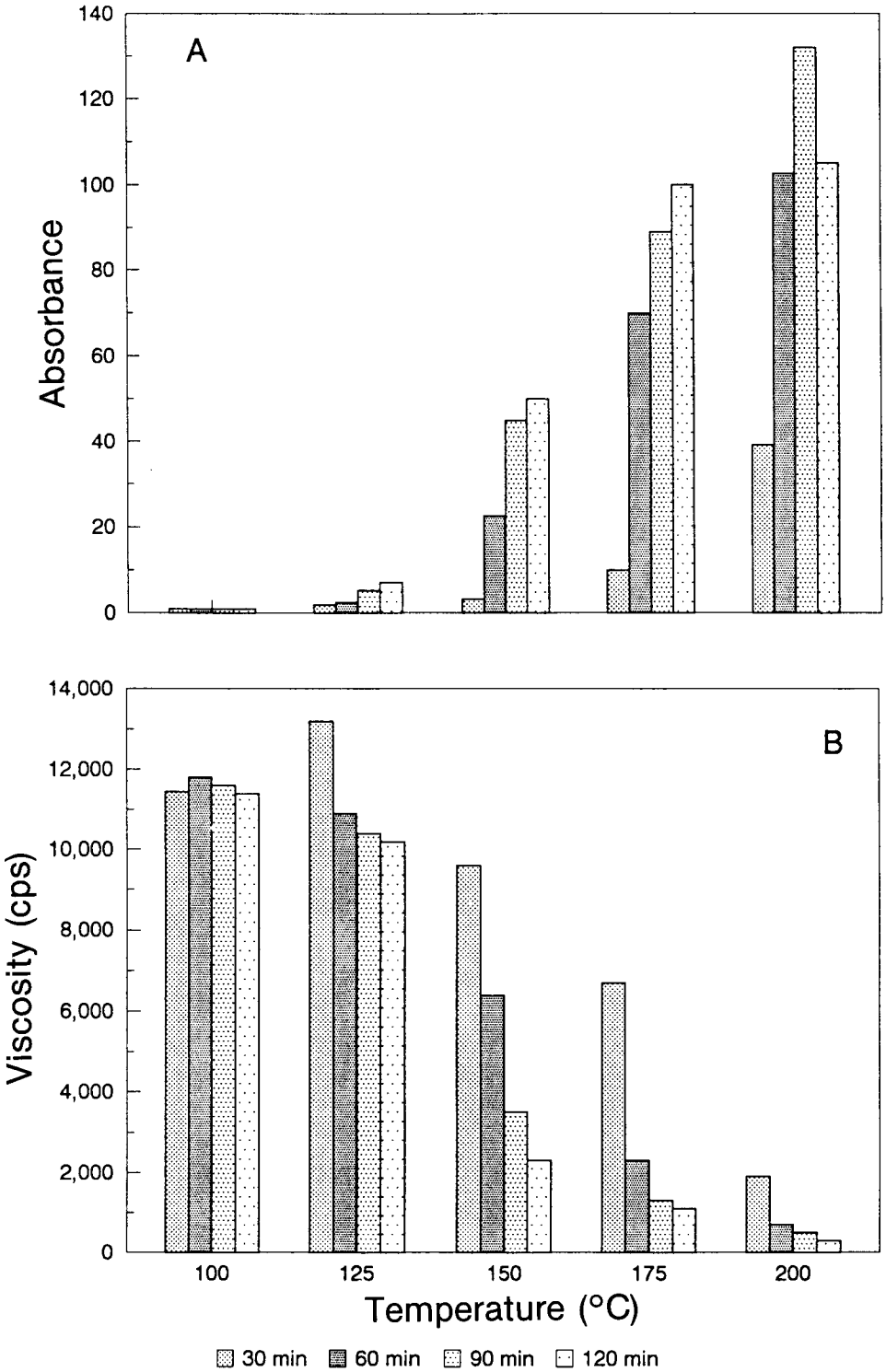

FIG. 1. A. Changes in the absorption of light at 350 nm by chloroform solutions of granular lecithin heated as a function of temperature and time. Samples were diluted as required to obtain absorption values that are presented on a relative scale of 0 to 140. B. Changes in the viscosity of liquid lecithin as a function of temperature and time where 200 g of granular lecithin was heated.

Fractionation. Thermalized lecithins were partially fractionated by separating the acetone-soluble (AS) and AI components. Acetone was added to the thermalized lecithin (4:1, vol/vol). The mixture was vortexed thoroughly 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.

HPLC. HPLC separation of the components of thermal-

TABLE 1

Ternary Solvent Program for the High-Performance Liquid Chromatography Analysis[a]

Time (min)	Ternary solvents		
	A (water)	B (isopropanol)	C (isooctane/tetrahydrofuran)
0	—	—	100
3	—	5	95
6	—	15	85
9	—	60	40
27	9	51	40
42	9	51	40
47	—	61	40

[a]See Experimental Procedures section for details.

ized and nonthermalized lecithin was conducted essentially by the method of Moreau *et al.* (7) in a Spectra Physics SP-8700 chromatograph (San Jose, CA) linked to a Spectra Physics SP-4270 Integrator. The chromatograph was equipped with a LiChrosorb Si 60 chrompak column (10 cm × 0.3 cm, 5 μm; Chromopack, Inc., Raritan, NJ) and a Varex Universal HPLC evaporative light-scattering detector (ELSDII) (Burtonsville, MD). Detector temperature was set at 100°C, exhaust temperature at 61°C and nitrogen pressure at 25 psi. A ternary solvent system, consisting of water (solvent A), isopropanol (solvent B) and isooctane/tetrahydrofuran (THF) (99:1, vol/vol) (solvent C), was used with the gradient program given in Table 1 at a flow rate of 0.5 mL/min. Solvent reequilibration between injections was accomplished by passing isopropanol/isooctane (60:40, vol/vol) through the column for 5 min and then solvent C for 35 min. Injection volume was 10 μL from a 2-mg/mL solution of the samples.

Fatty acid composition. The fatty acid components of the lecithins were analyzed as methyl ester derivatives by procedures described previously by Gandhi and Weete (8). Free fatty acids in the AS fractions of the thermalized lecithins were determined by standard titration procedures as described in the AOCS Official Method Ca 5a-40 (9).

Differential scanning calorimetry (DSC). Thermal analysis of granular lecithin was conducted with a TA Instruments thermal analyzer model 2000 (TA Instruments, Inc., New Castle, DE). Lecithn (10.2 mg) was placed in an aluminum sample holder, which was left uncovered while scanning from 100 to 300°C.

RESULTS

Discoloration. Browning of the lecithins increased as a function of time and temperature. Granular lecithin was heated for 30 to 120 min at 125 to 200°C, and the browning intensity was measured as absorbance at 350 nm in chloroform solution (0.5 g/10 mL). Discoloration increased with increasing temperature and with time at each temperature, beginning with amber to brown and then to dark brown-black. Maximum absorption was observed in samples heated at 175°C for 120 min and 200°C at 60 to 120 min. (Fig. 1A). Similar results were obtained with liquid lecithin and gum.

Changes in viscosity. When heated under conditions that improved the properties of lecithins as emulsifiers (see below), granular lecithin became a thick, dark brown-black tar-like material. Therefore, changes in viscosity were more easily demonstrated with the liquid form of lecithin than the granular form, which was heated at temperatures from 100 to 200°C for periods of 30 to 120 min each. Viscosity measurements were made after bringing the heated lecithin to room temperature. Temperatures up to 125°C had little effect on the viscosity of liquid lecithin when measured at 23°C regardless of heating time, but higher temperatures substantially reduced the viscosity with the magnitude of effect increasing with time (Fig. 1B). For example, the viscosity of 200 g of liquid lecithin heated for 90 min at 200°C, and then cooled to 23°C, was reduced 95% from that of the unheated material. Viscosity of the samples heated for 120 min was about 85% less than those for 30 min at either 175 or 200°C.

Improvements in properties as an emulsifier. Heating lecithin under certain conditions of time and temperature greatly improved its properties as an emulsifier for w/o emulsions. Initially, granular lecithin was heated for 60 min at temperatures from 100 to 250°C and used as the sole emulsifier for the preparation of emulsions containing 40% sucrose as the discontinuous phase. Increases in discontinuous phase-holding capacity and emulsion viscosity were used as evidence for the improvement in the properties as an emulsifier. Holding capacity increased for emulsions prepared with lecithin heated at 175°C and progressively increased by up to 77% for emulsions made with lecithin heated at 250°C (Table 2). Similarly, emulsion viscosity increased beginning with those prepared with lecithins heated at 175°C and increased sixfold for those containing samples heated at 200°C. Emulsions prepared with lecithin heated above 200°C were unstable, and viscosity could not be measured.

To determine the optimum heating time for the improvement of lecithin as an emulsifier, granular lecithin was heated at 180°C for 15 to 480 min. The holding capacity and viscosity increased 63% and eightfold, respectively,

TABLE 2

Properties of Emulsions Prepared with Granular Lecithin Treated Under Different Conditions of Temperature and Time[a]

Heating time (min) and temperature (°C)	Holding capacity (g)	Viscosity[b] (cps)
Temperature for 60 min		
100	101	2000
125	101	2000
150	101	2000
175	131	4000
200	149	12000
225	167	—
250	179	—
Time at 180°C		
15	95	2000
30	113	2000
60	131	6000
90	143	11000
120	155	16000
240	149	12000
480	155	10000

[a]Emulsions were prepared with 0.3 g granular lecithin heated as described, 5.7 g Klearol (Witco, Houston, TX), and 40% sucrose at the amount indicated.

[b]Viscosity measurements were made at 22–23°C and at 20 rpm with a Brookfield Viscometer (Brookfield Engineering Labs, Stoughton, MA) equipped with a #7 spindle.

for emulsions prepared with lecithin heated up to 120 min. Holding capacity did not increase further at longer heating times, and viscosity declined for emulsions prepared with lecithin heated for 240 min or more (Table 2).

To verify that the emulsification properties of various forms of lecithin can be improved by heating, several properties of emulsions prepared with thermalized granular, liquid and gum lecithins were compared with those containing the corresponding nonthermalized lecithins. The viscosities of emulsions might be expected to be directly correlated with the amount and quality of the surfactant used as the emulsifier. For example, the viscosity of emulsions prepared with 0.1 to 2.5 g thermalized granular lecithin increased from about 1800 cps to 14,000 cps (Fig. 2). Stability to gravitational settling increased with increasing emulsifier content where essentially no settling was observed after several days (Fig. 2).

The viscosities of emulsions prepared with thermalized lecithins were indeed substantially higher than those prepared with the corresponding nonthermalized lecithins. Emulsions prepared with thermalized granular, liquid and gum lecithins were 153, 61 and 200% higher than those made with the corresponding nonthermalized materials, respectively (Fig. 3A). In another example, it took 20 times more liquid lecithin in an emulsion for a viscosity that was approximately the same as that prepared with 0.5 g thermalized liquid lecithin (data not given).

The globules (particles) of emulsified aqueous phase were considerably smaller in the emulsions prepared with thermalized lecithins. For example, average particle diameters were 71, 87 and 63% smaller in emulsions prepared with thermalized granular, liquid and gum lecithins, respectively, than those in corresponding emulsions made with the nonthermalized materials (Fig. 3B).

Emulsion droplets on glass microscope slides were placed in a temperature- and humidity-controlled chamber with a high evaporative demand atmosphere, and water evaporation was measured as weight loss of the slides. After 4 h, emulsions prepared with nonthermalized granular, liquid and gum lecithins lost 30, 42 and 63% more water, respectively, by evaporation than the emulsions made with the corresponding thermalized materials (Fig. 3C).

In view of the above results, emulsions prepared with thermalized lecithins might be expected to be more stable than those made with nonthermalized lecithins. Therefore, stability to gravitational settling of the discontinuous phase was determined. When allowed to set undisturbed, emulsions prepared with thermalized granular, liquid and gum lecithins showed 47, 40 and 69% less settling than the corresponding emulsions containing the nonthermalized materials (Fig. 3D). Stability to centrifugation was also determined. For example, when centrifuged for 10 min, only 1000 rpm were required to break (phase separation) emulsions prepared with nonthermalized gum compared to 5000 rpm for the emulsion prepared with thermalized gum (data not given).

Fractionation and analysis of thermalized lecithin. Thermalized granular lecithin was separated into AI and AS fractions, which composed about 50% each of the original material (Fig. 4). However, the ratio of these fractions varied slightly with the batch of thermalized lecithin and with the form (granular, liquid or gum) of lecithins used (data not given). The AI fraction contained phosphatides

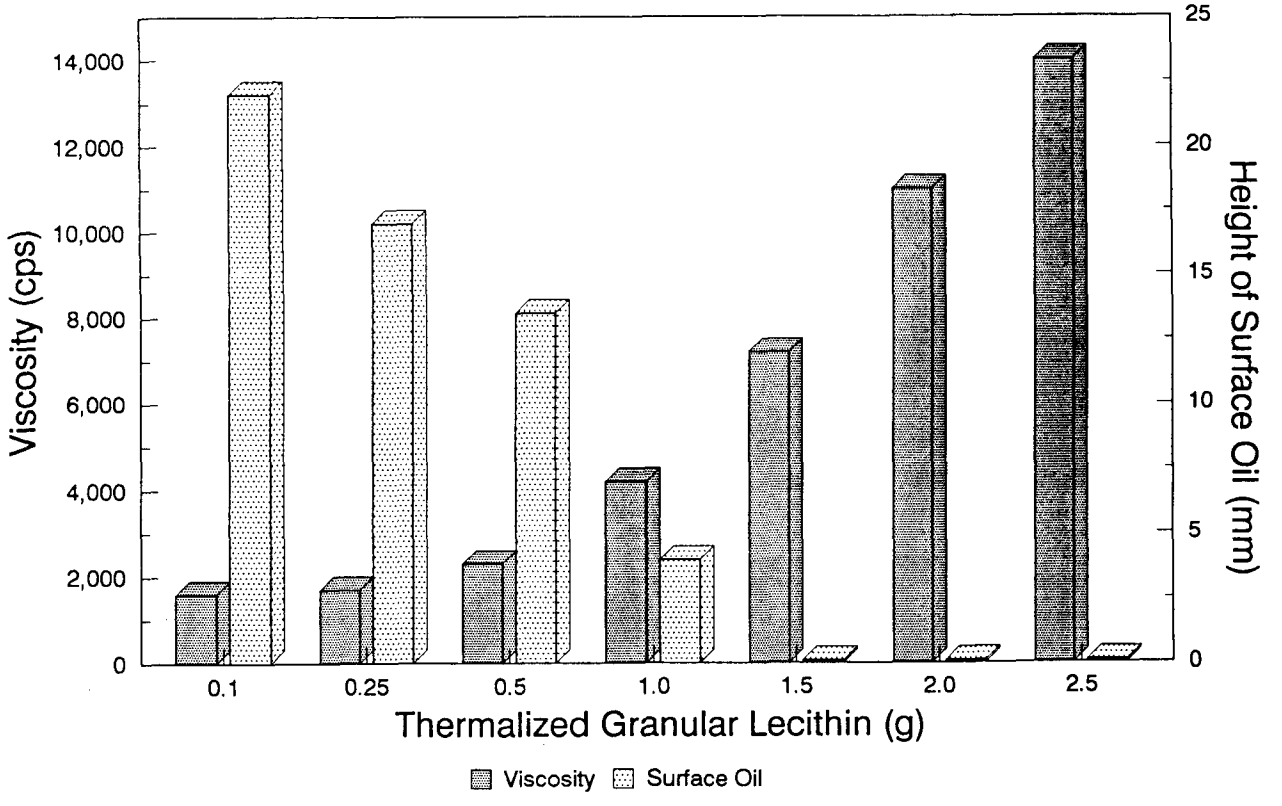

FIG. 2. Changes in emulsion viscosity and discontinuous-phase settling as a function of amount of thermalized granular lecithin used as the emulsifier. Measurements were made within 24 h after emulsion preparation.

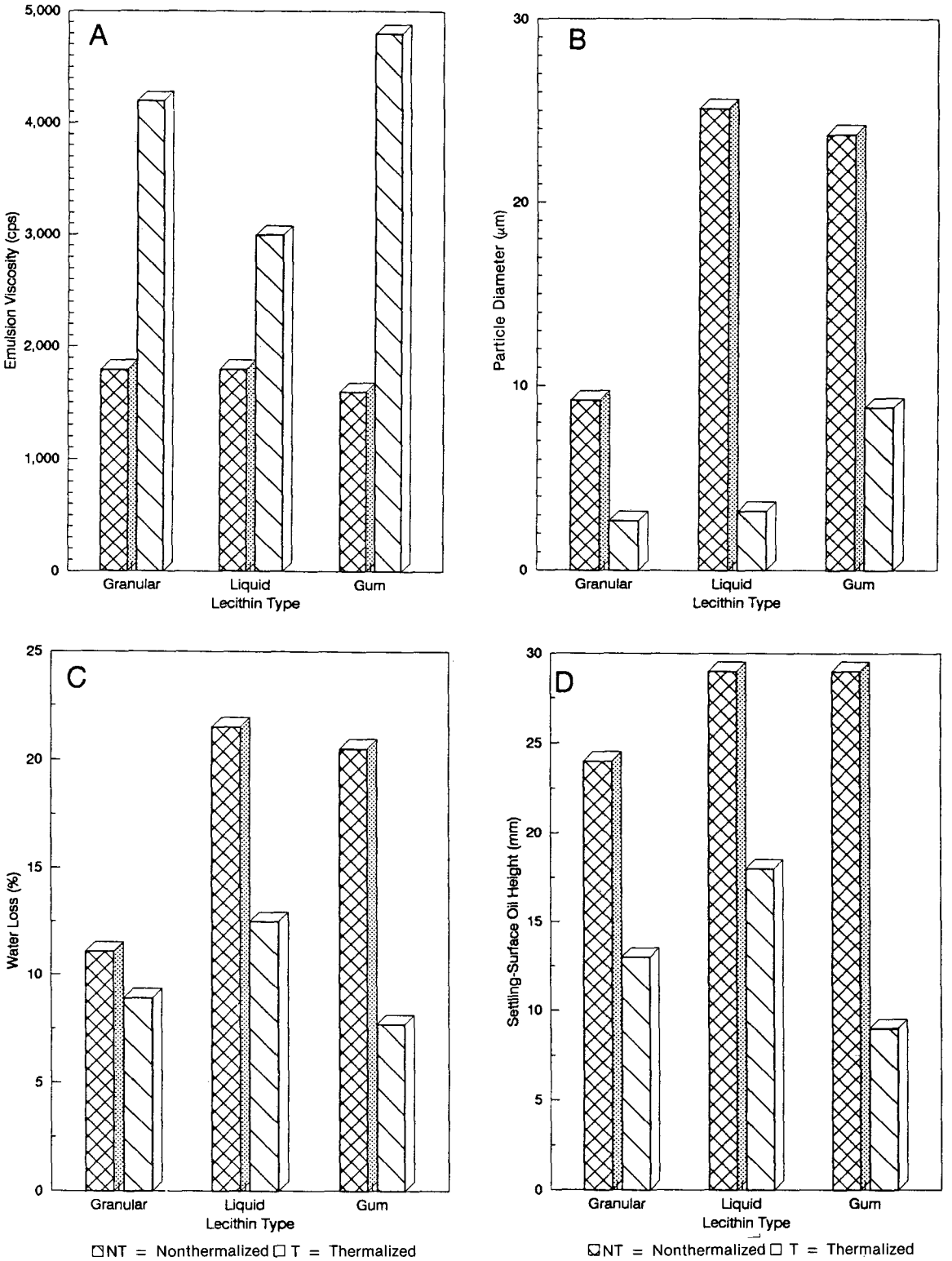

FIG. 3. A. Viscosity of emulsions prepared with thermalized or nonthermalized lecithins measured at 21°C. The lecithins were thermalized at 180°C for 60 min, and the emulsions contained 0.5 g thermalized or nonthermalized lecithin in 18 g Klearol (Witco, Houston, TX) B. Average diameters of globules (particles) of emulsified discontinuous phase in emulsions prepared with thermalized and nonthermalized lecithins. C. Water loss from emulsions prepared with thermalized and nonthermalized lecithins. D. Height of surface oil as a measure of gravitational settling of discontinuous phases of emulsions prepared with thermalized and nonthermalized lecithins.

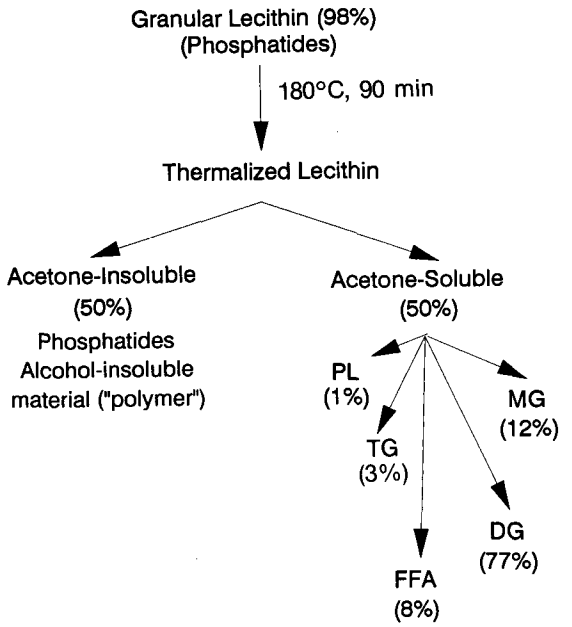

FIG. 4. Thermalization and fractionization of granular lecithin. PL = phosphatides, MG = monoglycerides, DG = diglycerides, TG = triglycerides, FFA = free fatty acids.

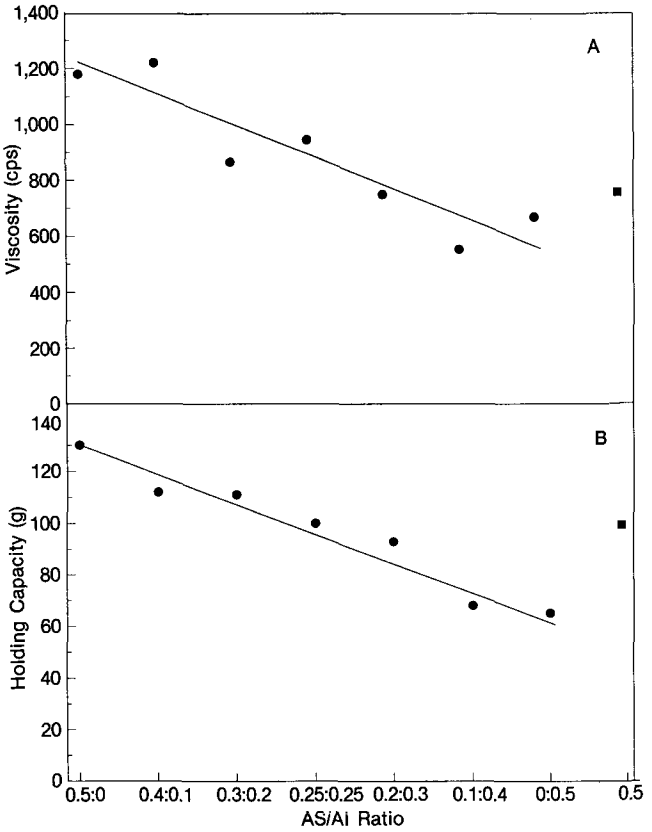

FIG. 5. (A). Viscosities and (B). discontinuous phase-holding capacities for emulsions prepared with acetone-insoluble (AI) and acetone-soluble (AS) fractions of thermalized granular lecithin alone and in various ratios. The square (■) represents the value for unfractionated thermalized lecithin. Except for the emulsifier, the composition of emulsions were as described in Figure 3A.

and an AI material believed to be a polymer (data to be reported separately). The AS fraction was a mixture of mono- (12%), di- (77%) and triglycerides (3%), free fatty acids (8%) and phosphatides (1%), according to HPLC and titration analysis. This is a substantial change in composition, considering that the granular lecithin used for thermalization contained 95–98% phosphatides. Similar trends were obtained with liquid lecithin and gum, but the final composition was influenced by the composition of the starting material for thermalization, e.g., thermalized liquid lecithin had a higher triglyceride content than the other forms of thermalized lecithin because the starting material contained 65% triglyceride.

Fatty acid compositions of the thermalized lecithins were essentially unchanged during thermalization. The fatty acid composition of thermalized granular lecithin was palmitic acid (20.4%), stearic acid (4.6%), oleic acid (7.3%), linoleic acid (60.6%) and linolenic acid (7.1%).

DSC analysis of granular lecithin indicated that there is considerable reaction activity in the 170 to 210°C range. There was a major endothermic peak in the thermogram from about 184.03 to 190.88°C, and endothermic shoulders at about 172, 180, 186 and 188°C that indicated additional reaction activity. Also, a major broad endothermic peak occurred from about 250 to 280°C.

Emulsification properties of AI and AS fractions. To assess the extent to which components of the AI and AS fractions contribute to the improved emulsification properties of thermalized lecithins, viscosities and discontinuous phase-holding capacities were determined for emulsions prepared with different ratios of AI and AS fractions. The viscosity and holding capacity for the emulsion, prepared with 0.5 g of the AS fraction as the emulsifier, were 58 and 30% higher, respectively, than the emulsion prepared with the unfractionated thermalized lecithin (Fig. 5). Both the viscosities and holding capacities of the emulsions decreased esentially linearly with increasing proportion of AI fraction in the emulsifier mix.

DISCUSSION

Heating for a sufficient time and temperature alters the appearance, composition and properties of lecithin. The expected discoloration, or browning, was observed when the three forms of lecithins used in this study were heated. Scholfield (10) has reviewed studies relating to the browning of lecithin due to heating, which is generally believed to be due to Maillard-type reactions involving amines (PE) and aldehydes (11,12). The browning reaction between acetaldehyde and phospholipid was proportional to the amount of PE added to the reaction mixture (13). In this study, the disappearance of PE corresponded to formation of the brown coloration of the heated lecithin (data to be reported separately). It has been suggested that the brown material formed during the heating of lecithin is a polymer that retains the structure of the original lecithin (14–17) and is formed by the reaction of aliphatic carbonyl compounds generated by the oxidation of unsaturated fatty acids, with PE (18).

Lecithin is an effective emulsifier for o/w emulsifions because its hydrophilic–lipophilic balance is in the 5–6

range, and it is widely used as such (4). However, in spite of browning, heating under certain conditions of time and temperature transforms the various forms of lecithins into improved emulsifiers for w/o emulsions (5,6). In addition to water or 40% sucrose used as the discontinuous phase in this study, thermalized lecithins can be used to effectively emulsify a wide range of organic and inorganic solutions in the pH range of 3 to 9 (5).

Reduced viscosity and improvement of lecithins as an emulsifier for w/o emulsions by thermalization can be explained by changes in composition, i.e., reduction in phosphatides and corresponding increase in neutral lipids, mainly diglycerides. Diglycerides result from the breakdown of the phosphatides, which is suggested by the results with thermalized granular lecithin, whereby the starting material contained 95–98% phosphatides. Thus, the reduced viscosity of lecithins by heating is probably due to both the reduction of polar components and increase of neutral lipid or relatively nonpolar substances. Likewise, improvement in the emulsification properties can be explained by the increase in diglycerides which are well known as emulsifiers for w/o emulsions.

Likewise, the dark-brown alcohol-precipitable material, which remains dissolved in the thermalized product, may alter the properties of the thermalized lecithin, but it is not a good emulsifier (data not given).

In summary, heating various forms of lecithins, from the most pure to the most crude, under certain conditions of time and temperature can improve their properties as emulsifiers for w/o emulsions. For example, batch heating of lecithins at 180°C for 90 min brings about the necessary compositional changes that are favorable for improvement in their emulsification properties. Changes in both the thermal properties and composition of granular lecithin correspond closely to the temperature required for improvement in properties as an emulsifier. Reaction activity in the 170 to 210°C range is also generally consistant with the thermal analysis of lecithin reported by Ross *et al.* (19). Optimum batch thermalization conditions may vary with the type or form and amount of lecithin used.

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REFERENCES

1. Schneider, M., in *Lecithins,* edited by B.F. Szuhaj, American Oil Chemists' Society, Champaign, 1989, pp. 111.
2. Kullenberg, F.W., in *Ibid.,* edited by B.F. Szuhaj, American Oil Chemists' Society, Champaign, 1989, pp. 238.
3. List, G.R., in *Ibid.*, edited by B.F. Szuhaj, American Oil Chemists' Society, Champaign, 1989, pp. 147.
4. Szuhaj, B.F. (ed.), *Ibid.,* American Oil Chemists' Society, Champaign, 1989, pp. 238.
5. Weete, J.D., and G. Griffin, U.S. Patent 4,943,389 (1990).
6. Weete, J.D., and G. Griffin, *Ibid.* 5,008,037 (1991).
7. Moreau, R.A., P.T. Asman and H.A. Norman, *Phytochemistry 29*:2461 (1990).
8. Gandhi, S., and J.D. Weete, *J. Gen. Microbiol. 137*:1825 (1991).
9. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* edited by David Firestone, American Oil Chemists' Society, Champaign, 1990, Method Ca 5a-40.
10. Scholfield, C.R., in *Lecithins* edited by B.F. Szuhaj, American Oil Chemists' Society, Champaign, 1989, pp. 11.
11. Scholfield, C.R., and H.J. Dutton, *J. Am. Oil Chem. Soc. 31*:258 (1954).
12. Corless, G.A., and L.R. Dugan, *Lipids 5*:846 (1970).
13. Suyama, K., and S. Adachi, *Agric. Biol. Chem. 43*:1981 (1979).
14. Tomioka, F., and T. Kaneda, *Chem. Abst. 82*:96613 (1975a).
15. Tomioka, F., and T. Kaneda, *Ibid. 82*:96614 (1975b).
16. Tomioka, F., and T. Kaneda, *Ibid. 86*:42075 (1977a).
17. Tomioka, F., and T. Kaneda, *Ibid. 86*:15426 (1977b).
18. Nakanishi, T., and K. Suyama, *Agric. Biol. Chem. 38*:1141 (1974).
19. Ross, R.A., R. Lemay and A.M. Takacs, *Can. J. Chem. Eng. 63*:639 (1985).

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