JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparative Evaluation of the Emulsifying Properties of Phosphatidylcholine after Enzymatic Acyl Modification

ANDERS F. VIKBJERG,*,† JEAN-YVES RUSIG,† GUNNAR JONSSON,‡ HUILING MU,† AND XUEBING XU†

BioCentrum-DTU and Department of Chemical Engineering, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark

The ability of enzymatically synthesized structured phosphatidylcholine (PC) containing caprylic acid to form and stabilize oil-in-water emulsions prepared with different triglycerides [medium chain triglycerides (MCT), soybean oil, and enzymatically synthesized structured lipids] was examined and compared with natural soybean PC and deoiled lecithin. Emulsions were prepared with varying oil and emulsifier concentrations. The particle size distribution, creaming stability, and viscosity were measured for the evaluation of the emulsifying properties. With an increase in the oil concentration, there was an increase in particle size, viscosity, and creaming layer. With an increase in the phospholipid (PL) concentration, there was usually a decrease in particle size and an increase in viscosity, where the emulsion stability was increased. General emulsions prepared with structured lipids resulted in smaller particle sizes as compared to MCT and soybean oil. Deoiled lecithin was able to increase the viscosity more significantly and give smaller particle sizes as compared to the other emulsifiers, thus producing more stable emulsions. However, in certain cases, structured PC was superior to deoiled lecithin and soybean PC. This observation was made for emulsions prepared with soybean oil or structured lipid at an oil/water ratio of 10:90. At an oil/water ratio of 30:70, the deoiled lecithin performed better as compared to the other PLs with all oil types. However, structured PC produced more stable emulsions as compared to natural soybean PC in MCT and soybean oil.

KEYWORDS: Emulsions; structured phospholipids; triglycerides; particle size; viscosity; stability

INTRODUCTION

Phospholipids (PLs) have been applied in both water-in-oil (w/o) emulsions and oil-in-water (o/w) emulsions for the production of foods, pharmaceuticals, and cosmetics. The ability of PLs to simultaneously interact with water and oil makes it an effective emulsifier. PLs help maintain stable emulsions between miscible liquids. The surface tension between the two liquids is decreased, which allows them to mix and form a stable heterogeneous dispersion. PLs in o/w emulsions are absorbed at the oil droplet surface forming a multilayer lamellar structure, whereas in w/o emulsions, PLs stabilize the emulsion by forming reverse micellar structures (*1*). For many industrial applications, crude PL products obtained from vegetable oil refining can be used directly; however, usually, it is desired to have some kind of purification. Crude vegetable lecithins contain 30−40% neutral lipids, predominantly triglycerides; the remainder consists of polar lipids, mainly a mixture of different PLs. To improve the handling of the highly viscous crude lecithin and to improve dispersability, industry commonly makes an acetone deoiling (*2*). Triglycerides (TAGs) dissolve in acetone, in contrast to the other more polar components of lecithin. With acetone extraction, PLs become more concentrated, which results in significantly lower dosage requirements and higher functionality.

There are various reasons for further purification of the PLs. Pure PLs have specific nutritional and pharmaceutical values, and specific PLs have dedicated surface active properties, which support the processing of emulsion stability and shelf life (*3*). Phosphatidylcholine (PC)-enriched lecithins have also been reported to deliver superior o/w emulsions capacity as compared to the standard deoiled product (*4*).

The molecular structure of PLs can be changed by either enzymatic or chemical means. The aim of these processes is to obtain tailor-made technological and/or physiological properties that differ from the natural substrate. Especially, enzymatic modification has gained increasing interest as enzymes can be used to modify PLs in a wide variety of ways. Commercial use to a large extent is known only with phospholipase A_2 (PLA_2) for partial hydrolysis to produce lyso-PLs. Partially hydrolyzed lecithin products possess improved emulsifying properties (*2*). The higher the degree of hydrolysis, the smaller the droplets are that are generated in a comparative emulsion process (*4*). Desired PLs with new physical and chemical properties can also be obtained by exchanging fatty acids in PLs. It has been

* To whom correspondence should be addressed. Tel: +45 4525 2614. Fax: +45 4588 4922. E-mail: afv@biocentrum.dtu.dk.
† BioCentrum-DTU.
‡ Department of Chemical Engineering.

10.1021/jf052665w CCC: $33.50
Published on Web 04/06/2006

Table 1. Fatty Acid Distribution in Oils and PLs

fatty acid composition (mol %)	triglycerides			PLs		
	MCT	soybean oil	structured lipid	deoiled lecithin	PC	structured PC
8:0	60.2		35.0			36.6
10:0	39.8					
14:0			4.2			
16:0		11.4	8.2	19.9	12.8	2.8
16:1			4.9			
18:0		3.4	0.8	4.4	3.9	0.7
18:1		22.8	10.6	10.1	9.4	5.8
18:2		55.6	1.3	59.2	65.8	48.8
18:3		6.9	4.9	6.6	8.1	5.3
18:4			5.1			
20:1			2.5			
20:4			0.7			
20:5			7.7			
22:5			1.1			
22:6			13.0			
degree of unsaturation (mol %)	0	85.2	51.8	75.7	83.3	59.9
average chain length	8.8	17.8	14.9	17.6	17.7	14.3

claimed that C_2-C_{18} saturated fatty acids may be incorporated to modify emulsification properties, to modify the physiological value, or to improve oxidation stability (*5*). For emulsions prepared with pure soybean PC, phase separation happens very rapidly; however, it is less pronounced for emulsions containing PC enriched with caproic acid (*6*). Several attempts have been made over the last two decades for the enzymatic acyl exchange; however, in general, the yield for these reactions has been low. During lipase-catalyzed acidolysis reactions, major byproduct formation usually occurs (*7*, *8*). It should be kept in mind that these byproducts are valuable products themselves as they also have wide applications in the same areas as the original material. These compounds can be purchased in purified form at different companies and are usually sold at considerably higher prices as compared to the natural PLs.

So far, there has been little effort to examine the emulsifying properties of structured PLs. The primary objective of the current study was to investigate the emulsifying characteristics of the synthesized structured PL containing caprylic acid using various triglycerides and to compare it with deoiled lecithin and purified soybean PC. Chain length and degree of saturation of oil and PLs are known to have a significant effect on the emulsion prepared (*9*). TAGs with considerable different fatty acid profile were thus selected for this study. These TAGs included MCT, soybean oil, and enzymatically synthesized structured lipids. Concerning emulsion properties, we determined the oil droplet size, the viscosity, and the stability.

MATERIALS AND METHODS

Materials. Deoiled lecithin (Sterninstant, PC-30) was donated by Stern Lecithin & Soja GmbH (Hamburg, Germany). PC (Epikuron 200, purity 93%) was obtained from Degussa Texturant Systems Deutschland GmbH & Co. KG (Hamburg, Germany). Caprylic acid (C8:0, purity 97%) was purchased from Riedel-de-Haen (Seelze, Germany). MCT (medium chain triglycerides) oil was purchased from Cognis Deutschland GmbH & Co. KG (Illertissen, Germany). Soybean oil was obtained from Aarhus United A/S (Aarhus, Denmark). Structured lipids were synthesized by acidolysis between fish oil and caprylic acid as previously described (*10*) and purified by thin film distillation (TFD). The fatty acid compositions of lipids and PLs used in the present study are presented in **Table 1**. Lipozyme RM IM, an immobilized sn-1,3 specific lipase from *Rhizomucor miehei*, was donated by Novozymes A/S (Bagsvaerd, Denmark). Membrane GR70PE (polysulfone membrane on a polyester support) was donated by Alfa Laval A/S (Nakskov, Denmark). Silica gel 60 (particles size 0.035−0.070 mm) was purchased from Fluka Chemie GmBH (Buchs, Switzerland). All solvents and chemicals used were of analytical grade.

Structured PC Synthesis. The reaction was carried out by an acidolysis reaction between PC and caprylic acid. The bioreactor was a jacketed stainless steel column ($l = 300$ mm, i.d. $= 21$ mm) packed with 37 g of Lipozyme RM IM ($l = 280$ mm).The substrate mixture was fed upward into the column, and the column temperature was held constant by a circulating water bath. The reaction substrates were pumped through the enzyme reactor by a pump from Fluid Metering Inc. (New York, NY). Reaction conditions were as follows: substrate volume, 100 mL; substrate ratio, 6 mol/mol caprylic acid/PC; reaction temperature, 55 °C; and water addition, 3% based on total substrate. The substrate was passed through the column for 48 h by recirculation. After reaction, the incorporation of caprylic acid was 37.4 mol %, and the PL distributions were 52, 28, and 20 wt % for PC, lysophosphatidylcholine (LPC), and glycerophosphorylcholine (GPC), respectively.

Removal of FFA from Reaction Mixture. A stirred dead-end ultrafiltration cell with magnetic stirrer (Millipore, Glostrup, Denmark) was used for the separation of free fatty acids (FFAs) from the reaction mixture. Pressurized nitrogen gas provided the driving force for the permeation. The cell's capacity was 300 mL with an effective membrane area of 40 cm^2. A GR70 PE flat sheet membrane was used for the separation of PLs and FFA. The membrane was soaked in ethanol prior to filtration. Membrane filtration was conducted at room temperature (20−25 °C), and the pressure was kept at 3 bar. The permeate was collected through a port beneath the membrane support. Initially, the cell was charged with 100 g of feed (30 w/w% reaction mixture in hexane), 30 g of permeate was collected, and a new 30 g of hexane was added. The experiment was thus performed as a discontinuous diafiltration. The addition of more solvent was done in order to improve the permeation rate, since the flux was seen to continuously decrease with concentration factor. Twelve batches of 30 g of permeate were collected with subsequent addition of hexane. Ninety-two percent of the FFAs were removed by this procedure. The diafiltration was conducted three times. The PL distribution was not changed by the diafiltration, and further purification was required in order to have separation of PC from other PL constituents.

Separation of PC from Other PL Species. To separate PC from LPC, GPC, and small amounts of FFAs after ultrafiltration, column chromatography was applied. The column was packed with 30 g of silica. The PL species were eluted with two different solvent systems. Chloroform/methanol/water (65:35:5 v/v/v) was used to elute FFA and PC, and methanol/water (90:10 v/v) was used to elute LPC and GPC. Fractions of 10 mL were collected. Fractions were analyzed, PC-containing fractions were pooled, and the solvent was evaporated followed by lyophilization. The purity of the structured PC was 92% after purification. The fatty acid composition of the structured PC can be seen in **Table 1**.

Emulsion Preparation. Components of the emulsion except water were weighed according to the ratio (10 or 30% of oil and 0.5 or 2% of PLs) and were heated to 60 °C in a beaker with gentle stirring until the PLs were completely dissolved in the oil. Then, the water was weighed and dispersed into the oil phase. The mixture was homogenized for 10 s at 13500 rpm with an ultraturrax T25 (Janke & Kunkel GmbH & Co., Staufen, Germany).

Fatty Acid Composition Analysis. Fatty acid methyl esters (FAMEs) were prepared by methylation and analyzed on a HP6890 series gas−liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector (FID), as described elsewhere (*7*).

FFA Content. This was determined by AOCS official methods (*11*). FFAs were 0.11, 0.05, and 0.74% for MCT, soybean oil, and structured lipids, respectively.

Microscopic Examination. An Optiphot light microscope (Olympus Co., Tokyo, Japan) was used to observe the structure of the emulsions. The emulsion samples were smeared on microscope slides and observed at 20× magnification.

Particle Size Distribution. Particle size analysis was performed with a laser diffractometer Mastersizer 2000 (Mastersizer S, Malvern Instruments, Malvern, United Kingdom) using standard optical parameters. Each sample was measured in triplicate. The surface mean diameter (Sauter diameter), $D[3,2] = \sum d^3/\sum d^2$, and the span, particle diameter at 90% cumulative size − particle diameter at 10% cumulative size, were calculated. The Sauter diameter is the equivalent spherical diameter by surface area per unit volume to the full distribution, i.e., the particle diameter that has the same specific surface as that of the full distribution. The span provides a measure between the points of distribution and therefore signals the quality of the distribution. A small span indicates a narrow size distribution.

Rheological Properties. Viscosity was measured using a concentric cylinder bob cup CC25 measuring system by Stresstech rheometer (Version 3.8, Reologica Instruments AB, Sweden). A constant temperature of 25 °C was maintained during the measurements with a circulatory water bath. Shear stress was increased progressively from 0.5 up to 2 Pa in 20 logarithmic steps with a continuous upward sweep direction. The viscosity was determined as the slope of shear stress vs shear rate curve.

Determination of Oil Density. Masses of the oils were determined by weighing into a 100 mL volume. The density was then calculated as the mass/volume. The densities of the oils were 0.94, 0.92, and 0.93 g/mL for the MCT, the soybean oil, and the structured lipids, respectively.

Emulsion Stability. For each emulsion, two test tubes were filled with 10 mL of the emulsion and closed with a cap. Samples were stored at 2 °C. The height of the total system and the height of cream separated out at the top were measured at 2, 4, 8, 16, and 32 days. A larger value of the cream layer was an indication of a more stable emulsion. If no macroscopic changes were observed, the creaming volume percentage was set at 100.

Statistics. Differences in particle size distribution, viscosity, and emulsion stability were determined by one-way analysis of variances, where 95% confidence intervals, that is, $P \leq 0.05$ significance level, were calculated from pooled standard deviations using software Microsoft Office Excel 2003 (Microsoft Corp., Redmond, WA). Data are expressed as the average of at least double determinations.

RESULTS AND DISCUSSION

The stability of an emulsion is controlled by interfacial surface forces, the size of the disperse phase droplets, viscous properties of the continuous phase, and the density difference between the two phases. To have a good o/w emulsion, the surfactant should orient most of the molecule in the water dispersion medium to maximize the reduction in the interfacial tension. An HLB system (hydrophilic/lipophilic balance) is often used for the selection of emulsifiers and is a measure of the surfactant's preference for oil or water, with the higher the number corresponding to a greater hydrophilicity-to-lipophilicity ratio. A high HLB number is preferred for o/w emulsions. The HLB value for purified soybean PC is approximately 7, and for deoiled soybean lecithin, it is 4 (*2*). For o/w emulsions, it would thus be expected that purified soybean PC would result in more stable emulsions as compared to deoiled soybean lecithin. With the enzymatic exchange of the long chain fatty acids with medium chain fatty acids in the pure soybean PC, it would become more hydrophilic and thus have an improved function as an emulsifier in o/w emulsions.

Emulsion instability is a complex process, which involves different mechanisms contributing to the transformation of a uniformly dispersed emulsion into a totally phase-separated system. Stokes' law equation gives a quantitative indication of the physical factors that influence the stability of an emulsion:

$$\upsilon = \frac{2r^2\,\partial\,\rho\,g}{9\eta} \qquad (1)$$

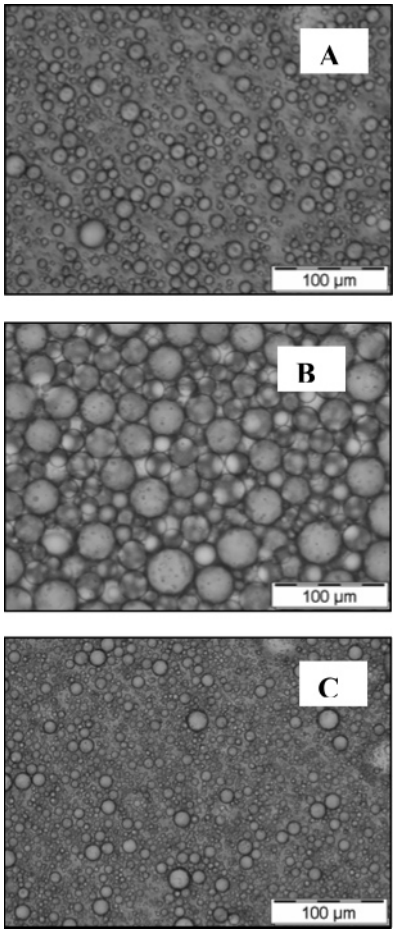

Figure 1. Microscopic view of emulsion with 10% oil and 2% structured PC: (**A**) MCT, (**B**) soybean oil, and (**C**) structured lipid.

where υ is the rate of phase separation, r is the radius of the particles, $\partial\rho$ is the difference in density between the two liquids, g is the gravity, and η is the viscosity of the medium. The stability of the emulsion is enhanced by small settling velocities of the dispersed oil particles. From the equation, it can be seen that especially the particle size is of critical importance as it occurs as a squared term. Emulsions are also more stable when density differences are small and when the viscosity of the medium is high. For the evaluation of the PL-stabilized emulsions, the particle size of the dispersed droplets, the viscosity, and the oil density were determined in order to calculate a theoretical separation phase rate based on these physical factors. Furthermore, the creaming stability of the emulsions was followed during cold storage (2 °C).

Microscopic Examination. The structures of all of the PL-stabilized o/w emulsions were similar. All of the emulsions prepared showed round droplets uniformly dispersed in the system. Structures of selected emulsions observed with a microscope are presented in **Figures 1** and **2**. Particle sizes of o/w emulsions are known to depend on various factors such as dispersed oil and its ratio to the continuous water phase, the emulsifier and its concentration, and the method of emulsion preparation. With all of the emulsions, there could be observed particles with varying particle sizes. In general, the particle size increased with an increase in oil concentration and with a decrease in PL concentration. It has previously been reported that ≥0.5% (wt/vol of the continuous phase) lecithin is required in order to have spherical structures in the emulsion (*12*). At lower lecithin concentrations, oil droplets can be observed in the aqueous continuous phase.

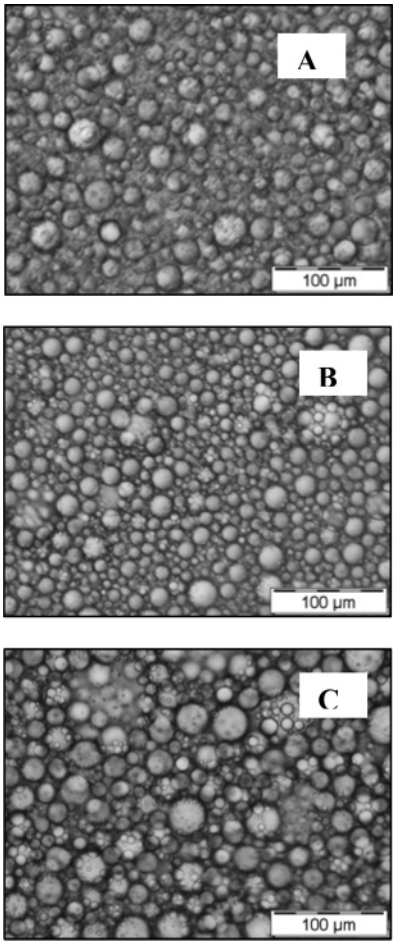

Figure 2. Microscopic view of emulsion with 30% soybean oil and 2% emulsifier: (**A**) deoiled lecithin, (**B**) PC, and (**C**) structured PC.

Particle Size Distribution. The particle size analysis confirmed the microscopic examination. The particle size varied within each emulsion prepared. In general, the particle size decreased with an increase in PL concentration and a decrease in oil concentration (**Figures 3** and **4**). When the Sauter diameter decreased, there was usually also a decrease in the span. Increasing the PL concentration reduced the size of large vesicles and had little effect on small emulsified droplets. Emulsion with an o/w ratio of 10:90 generally showed a smaller particle size and span with structured lipids as compared with emulsions prepared with MCT and soybean oil. In most cases, the largest particle size and span was observed for emulsions prepared with soybean oil. The largest particles were observed for emulsions prepared with PC and soybean oil, and the smallest particles were observed for emulsions with deoiled lecithin and structured lipids. Emulsions prepared with structured PCs usually had a larger particle size and span as compared to deoiled lecithin, except with structured lipids where there was no significant difference in particle size. Structured PCs gave smaller particles and spans than soybean PC in emulsions prepared with soybean oil at low PL concentrations and emulsions prepared with structured lipids at high PL contents.

For emulsions with an o/w ratio of 30:70, the smallest particles could in general also be observed for emulsions containing structured lipids. The largest particle size was found for emulsions prepared with deoiled lecithin and structured lipids; however, this was not the emulsion with the largest span, which was found for emulsions prepared with structured PCs and soybean oil. At low concentrations of deoiled lecithin, the

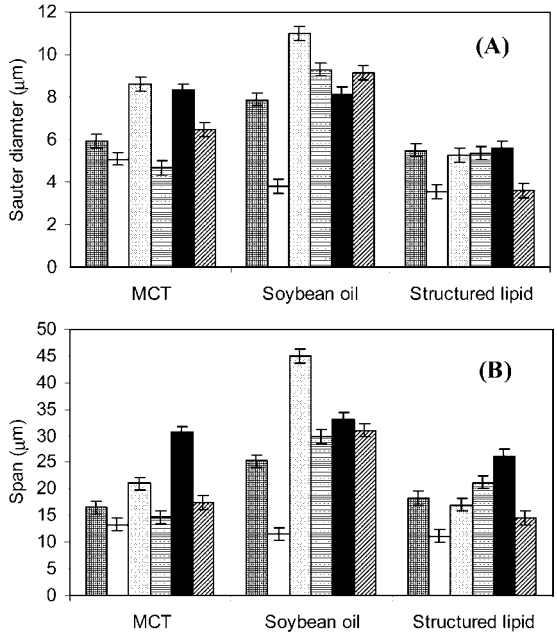

Figure 3. Particle size distribution of PL-stabilized emulsions with an o/w ratio of 10:90: (**A**) Sauter diameter and (**B**) span. Key: 0.5% deoiled lecithin, checked bar; 2% deoiled lecithin, white bar; 0.5% soybean PC, dotted bar; 2% soybean PC, horizontally striped bar; 0.5% structured PC, black bar; and 2% structured PC, diagonally striped bar. Bars indicate a 95% confidence interval based on pooled standard deviation.

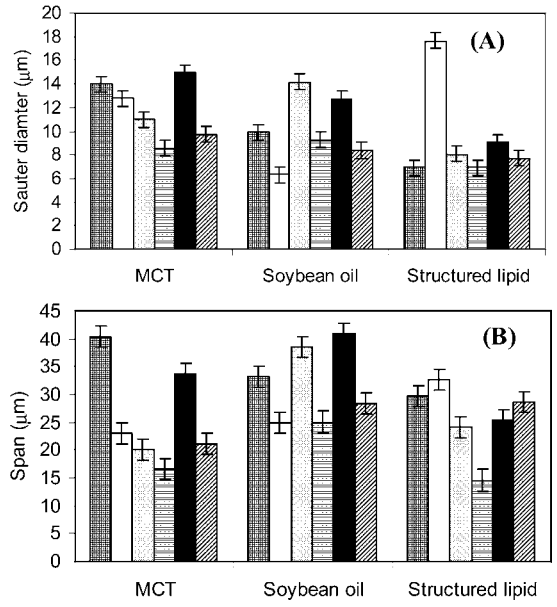

Figure 4. Particle size distribution of PL-stabilized emulsions with an o/w ratio of 30:70: (**A**) Sauter diameter and (**B**) span. Key: 0.5% deoiled lecithin, checked bar; 2% deoiled lecithin, white bar; 0.5% soybean PC, dotted bar; 2% soybean PC, horizontally striped bar; 0.5% structured PC, black bar; and 2% structured PC, diagonally striped bar. Bars indicate a 95% confidence interval based on pooled standard deviation.

largest span was found for emulsions prepared with MCT followed by soybean oil and structured lipids, respectively. However, when the PL concentration was increased to 2%, the reverse was observed. Determining which oil will result in the smallest particle size distribution in the emulsion is therefore highly dependent on the PL concentration. With soybean PCs used as an emulsifier, soybean oil gave the largest particle size and span. With the structured PC, the largest particle size was

found in MCT followed by soybean oil and structured lipids. Structured PCs produced smaller particles in emulsions prepared with soybean oil at both low and high PL contents as compared to soybean PC; however, the span was higher for the structured PCs. At a high concentration of PL, the structured PCs gave smaller particle sizes as compared to the deoiled lecithin in emulsions prepared with MCT and structured lipids.

Characteristics of various saturated and unsaturated PC used for emulsifying MCT have previously been reported (*9*). Particle sizes were shown to be influenced by the length and degrees of unsaturation of the acyl chain of the PC. The mean diameter of emulsion droplets increases as the number of carbons in the acyl chain of PC and TAG increases. The particle size also tends to increase with increased saturation degree (*9*). PCs with 6−10 carbons in their acyl group were better to form stable o/w emulsions, because these PCs are still able to form bilayer structures and also have stronger hydrophilic properties (*9*). TAGs having long acyl chains are highly lipophilic and, thus, more difficult to emulsify. Soybean oil also has a longer average chain length as compared to the other oils used in this study (**Table 1**), which could explain why it has a larger particle size in general. Soybean oil also has the highest degree of unsaturation. However, the results of this study indicate that in order to have small particles the oil should rather have a shorter chain length as compared to a high degree of unsaturation. On the basis of Stokes law equation (eq 1), it would be expected that emulsions prepared with structured lipids would have increased stability as compared to emulsions prepared with other oils since the particle sizes generally are smaller. Only in soybean oil, the particle size was smaller for structured PCs as compared to PCs. Exchange of long chain fatty acids with medium chain fatty acids in PC does not necessarily result in smaller particle size in the emulsions as it also seems to depend on the oil in use.

Rheological Characteristics. The rheological parameters are reflections of interactions and of the particle structure. The presence of lecithin changes the attractive forces between the particles; therefore, the rheological characteristics of the emulsion are affected by the type and amount of emulsifier. Shear stress and viscosity values of an emulsion change as the shear rate is increased (*13*). Shear stress is inversely proportional to viscosity. Viscosity was generally higher for the emulsion prepared with deoiled lecithin as compared to other emulsifiers used (**Figure 5**). With an increase in PL and oil concentration, the viscosity increased. Viscosity was dramatically increased for emulsions containing soybean oil and structured lipids at an o/w ratio of 30:70. Viscosity was also significantly higher for structured PCs as compared to soybean PCs in emulsions containing structured lipids with high PL content at an o/w ratio of 10:90. In other emulsions, structured PCs gave similar or lower viscosities.

Emulsion Stability. Creaming occurs when dispersed particles either settle or float with respect to the continuous phase and when either the lower or the upper portion, respectively, becomes more opaque or creamier. Creaming volume in the emulsions during cold storage is shown in **Tables 2** and **3**. All of the emulsions exhibit a tendency to creaming, except emulsions prepared with 10% MCT and 2% deoiled lecithin. During 32 days of storage, creaming was not observed for this emulsion. Higher PL concentrations usually increased the cream volumes and slowed the creaming process. Initially, all emulsions seemed stable by visual inspection as there was not observed any phase separation immediately after preparation. In most cases, emulsions remained opaque at the base of the sample, while a concentrated cream layer developed at the top of the sample. No oil separation was observed during the 32 days that the emulsions were followed. Destabilization kinetics of the different emulsions were very different. For some emulsions, the phase separation was not evident until prolonged storage time. Many of the emulsions, however, separated in two phases within 2 days. In some cases, the cream layer changed little over time, and in other cases, dramatic changes could be observed during storage. With low PL concentration (0.5%), phase separation usually happened fast and the cream layer changed very little over time. In emulsions prepared with soybean oil, the phase separation also happened within 2 days.

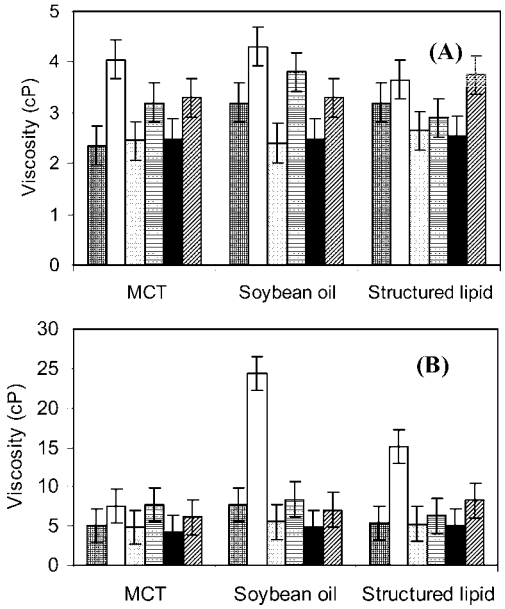

Figure 5. Viscosity of PL-stabilized emulsions with an (**A**) o/w ratio of 10:90 and an (**B**) o/w ratio of 30:70. Key: 0.5% deoiled lecithin, checked bar; 2% deoiled lecithin, white bar; 0.5% soybean PC, dotted bar; 2% soybean PC, horizontally striped bar; 0.5% structured PC, black bar; and 2% structured PC, diagonally striped bar. Bars indicate a 95% confidence interval based on pooled standard deviation.

Table 2. Stability under Cold Storage (2 °C) for PL-Stabilized Emulsions with an o/w Ratio of 10:90 and Calculated Phase Separation Rate

			creaming volume (%)[a]					
oil	emulsifier	emulsifier content (%)	day 2	day 4	day 8	day 16	day 32	phase separation rate (μm s^{-1})[b]
MCT	deoiled lecithin	0.5	20	21	23	24	26	0.46
		2	100	100	100	100	100	0.20
	PC	0.5	15	15	15	15	15	0.92
		2	5	7	9	8	10	0.21
	structured PC	0.5	21	19	18	18	15	0.84
		2	43	42	42	41	35	0.38
soybean oil	deoiled lecithin	0.5	17	17	17	17	16	0.59
		2	12	15	15	15	18	0.10
	PC	0.5	12	13	13	13	14	1.54
		2	13	14	14	14	14	0.69
	structured PC	0.5	21	19	19	18	18	0.81
		2	39	38	38	35	32	0.77
structured lipid	deoiled lecithin	0.5	24	24	24	21	20	0.29
		2	100	100	22	23	23	0.10
	PC	0.5	16	16	17	17	18	0.32
		2	13	14	14	14	15	0.30
	structured PC	0.5	23	21	21	19	15	0.38
		2	100	100	100	100	16	0.11

[a] The 95% confidence limit was ± 2.0 (based on pooled standard deviation). [b] Calculated according to Stokes' law equation (eq 1).

Table 3. Stability under Cold Storage (2 °C) for PL-Stabilized Emulsions with an o/w Ratio of 30:70 and Calculated Phase Separation Rate

oil	emulsifier	emulsifier content (%)	creaming volume (%)[a] day 2	day 4	day 8	day 16	day 32	phase separation rate (μm s^{-1})[b]
MCT	deoiled lecithin	0.5	47	48	51	51	49	1.18
		2	94	94	90	90	90	0.66
	PC	0.5	45	45	43	43	42	0.77
		2	51	49	46	44	44	0.29
	structured PC	0.5	41	39	39	39	39	1.60
		2	100	100	53	52	45	0.47
soybean oil	deoiled lecithin	0.5	53	49	48	46	46	0.38
		2	100	68	63	59	56	0.05
	PC	0.5	45	45	43	42	42	1.10
		2	48	46	45	42	45	0.31
	structured PC	0.5	43	43	41	41	41	1.03
		2	75	68	63	60	57	0.3
structured lipid	deoiled lecithin	0.5	53	53	50	48	47	0.27
		2	92	84	74	62	56	0.63
	PC	0.5	56	54	51	50	50	0.38
		2	100	100	100	55	54	0.23
	structured PC	0.5	44	42	41	41	40	0.50
		2	100	45	43	43	41	0.22

[a] The 95% confidence limit was ± 3.2 (based on pooled standard deviation). [b] Calculated according to Stokes' law equation (eq 1).

During the whole storage time, the cream layer volume was much higher for emulsions prepared with structured PCs as compared to the other emulsions prepared with 10% soybean oil. Similar phenomena were observed for the MCT where structured PCs resulted in a much higher cream layer as compared to soybean PC. With structured lipids, the structured PC was able to maintain a stable emulsion for at least 16 days. After day 32, the cream layer was similar to emulsions prepared with PC.

Emulsions prepared with 30% oil showed considerably higher cream layers as compared to emulsions prepared with 10% oil (**Table 3**). Some of these emulsions were able to maintain a stable emulsion for a few days; however, after 16 days, they had all separated into two phases. At 32 days of storage, the highest cream layer was observed for 2% deoiled lecithin with MCT. With soybean oil, the largest cream layer was observed for emulsions prepared with structured PCs, and with structured lipids, it was deoiled lecithin. For some emulsions, phase separation happened fast and only changed slightly over time. Other emulsions were shown to decrease gradually over time, as seen for emulsions prepared with soybean oil and structured lipids with 2% deoiled lecithin. This was also observed for the structured PC in soybean oil. In most cases, emulsions prepared with PC had rapid phase separation; however, with emulsions containing structured lipids, it was possible to stabilize the emulsion for more than 8 days. Emulsions prepared with MCT and structured lipids were most stable when prepared with deoiled lecithin; however, soybean oil was more stable with structured PCs. The cream layer volume at 32 days was shown to vary greatly among the emulsions prepared. In general, the cream layer volume was high for emulsions prepared with deoiled lecithin. Previously, it has been reported that 10% structured lipid o/w emulsions were not stable to creaming when 0.25−1% deoiled soybean lecithin was used (*14*). With low concentrations of pure PC in o/w emulsions, oil separation also occurred immediately with soybean oil (*1*). Pan et al. reported that emulsions containing 0.5% sunflower lecithin presented a faster creaming process than systems containing 1.0% for emulsions prepared with 30% sunflower seed oil (*12*). At levels of 2.5 and 5%, clarification was hardly detectable, which was explained by vesicles that occluded a greater part of the sample. The ability of PLs to stabilize emulsions has been known for decades; however, the stabilization mechanisms remain controversial. Rydhag and Wilton stated that the effectiveness of lecithin was mainly determined by the proportion of negatively charged PLs (*1*). However, Van der Meeren et al. reported that the stabilizing effect of PC-enriched lecithin was better as compared to deoiled lecithin (*15*). Deoiled lecithin is highly negatively charged, whereas the PC-enriched lecithin is rather zwitterionic. We observed that in most cases deoiled lecithin was better in stabilizing the emulsion as compared to soybean PC. However, at an o/w ratio of 30:70, the PC was able to stabilize emulsions with structured lipids for longer times as compared to the deoiled lecithin. Therefore, it seems difficult to make clear conclusions about which PL gives better stability as it depends on the particular formulations.

Applying Stokes' law equation (eq 1) with the measured data, the theoretical phase separation rate was calculated (**Tables 2** and **3**). The calculated phase separation rate was higher for emulsions prepared with 0.5% PL as compared to emulsions prepared with 2%. The phase separation rate was generally lower in emulsions prepared with structured lipid, which indicates that the presence of this oil may help increase its stability by physical means. Because the radius has a squared term in Stokes law equation, the calculated phase separation rate will be highly correlated to the particle size. Emulsions prepared with 10% soybean oil and 2% deoiled lecithin had a low calculated phase separation rate, however, already after 2 days the emulsion had separated. Emulsions prepared with 10% structured lipid and 2% structured PC also had a low phase separation rate; however, this emulsion was able to remain stable up to 16 days. For emulsions with an o/w ratio of 30:70, the phase separation rate were slowest in emulsions prepared with soybean oil and deoiled lecithin. This emulsion was stable for 2 days, and the cream volume was seen to gradually decrease during cold storage. In some cases, the emulsion had a higher phase separation rate as compared to other emulsions prepared; however, it was still able to maintain stability, e.g., emulsion prepared with 30% MCT and 2% deoiled lecithin. With an increase of PL concentration, the particle size usually become smaller, the viscosity increases, and also, the stability increases. However, it seems difficult to compare between different oils and PL compounds, as different kinds of interactions may occur. The tendency that can be observed for 10% oil emulsion may be completely different when the oil concentration is increased. With higher o/w ratios (0.4−0.6) and more than 0.5% PC, it was reported that emulsions became very viscous and did not separate spontaneously within 24 h (*1*). The emulsion was mechanically stabilized with respect to creaming while coalescence was not prevented. Coalescence is observed as the increase of emulsion droplets over time. Oil-in-water emulsions having the same lecithin concentration but varying levels of oil and water are not as good as w/o emulsions (*16*). A better emulsion stability is associated with emulsions having a high ratio of o/w (>60:40). With low oil concentrations, the destabilization occurs rapidly, whereas with higher oil concentrations, the oil separation is slowed. The stability of the emulsion is not only determined by the physical factors according to Stokes' law but is also related to interfacial forces within the emulsion. PL packing type (spontaneous curvature) is known to affect both the phase behavior of microemulsions and the coalescence energy barrier for the macroemulsions and has been correlated with the stability of emulsions formed (*17*). As a general trend, o/w emulsions become more stable as the spontaneous curvature increases. The

spontaneous curvature is known to increase with decreasing temperature, higher PC content, higher degree of saturation of the PL chains, long chain oils, and low penetration of oil into the surfactant "brush" (*17*).

The findings of this study give some useful information on the PL-stabilized o/w emulsions. At an o/w ratio of 10:90, the structured PC was shown to be superior to the other emulsifiers tested when soybean oil and structured lipids were used. Furthermore, structured lipids had interesting emulsifying properties as they were able to produce smaller particle sizes as compared to MCT and soybean oil, which were more commercially excisable. Deoiled lecithin and structured PC seemed to have the ability to stabilize all oils tested for several days. Soybean PC only produced stable emulsions with structured lipids. The optimal concentration of PL and oil will depend on the actual application. In the current study, we used high-shear homogenization; however, in order to increase the stability, high-pressure homogenization may be applied instead as smaller particles can be obtained (*6*). It is apparent that PLs are surface-active components, which through processes such as deoiling, fractionation, and modification, can be tailored to special applications. In the food industry, the deoiled lecithin is preferred over the purified PLs, since the price is considerably lower. In the pharmaceutical and cosmetic industry, further chromatographic purification exists and often different chemical modifications of PLs are made, e.g., hydroxylation, acetylation, and hydrogenation. Modified PLs with altered emulsifying and dispersing properties extend the application range of PLs in these areas. With enzymatic modification, it would be able to produce PLs with defined fatty acid compositions, which can be targeted for specific applications in foods, pharmaceuticals, and cosmetics. In this study, the substrate was produced from purified PC; however, acyl exchange can also be done for deoiled lecithin as previously demonstrated (*18*).

ABBREVIATIONS USED

FAMEs, fatty acids methyl esters; FFA, free fatty acids; FID, flame ionization detector; GC, gas chromatography; GPC, glycerophosphorylcholine; HLB, hydrophilic/lipophilic balance; LPC, lysophosphatidylcholine; MCT, medium chain triglycerides; o/w, oil-in-water; PC, phosphatidylcholine; PL, phospholipid; TAG, triacylglyceride; TFD, thin film distillation; TLC, thin-layer chromatography; w/o, water-in-oil.

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Received for review October 27, 2005. Revised manuscript received February 27, 2006. Accepted March 16, 2006. Financial support from the Danish Technical Research council (STVF) and the Center for Advanced Food Studies (LMC) is acknowledged. The Socrates program is acknowledged for the partial support of J.-Y.R. during his training period.

JF052665W